UNITED NATIONS
INTERNATIONAL DRUG CONTROL
PROGRAMME

RECOMMENDED METHODS FOR TESTING OPIUM, MORPHINE AND HEROIN

MANUAL
FOR USE BY NATIONAL
DRUG TESTING LABORATORIES

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Vienna

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Laboratory Section

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INTRODUCTION

During the last two decades, there has been a considerable increase in the number of substances newly placed under international control. At the same time, seized quantities of drugs already under control have also shown an alarming increase in certain regions. This new situation, involving a significantly increased variety of drugs and amounts seized, presents a challenge both to national law enforcement authorities and to the scientific staff of forensic laboratories.

Today, analysts must be able to analyze a wide range of substances and preparations, and use faster, more accurate and more specific methods for identification and analysis in order to cope with the increased analysis turnover and the requirements of stiffer national drug laws. In addition, the international character of drug trafficking requires the timely exchange of analytical data between laboratories and law enforcement authorities at the national, regional and international levels. For these reasons, UNDCP's Laboratory Section has since the early eighties vigorously pursued a programme of harmonization and establishment of recommended methods of testing for national drug testing laboratories.

The Commission on Narcotic Drugs noted at various occasions with satisfaction, the progress of such efforts and in emphasizing the importance of the convening of expert group meetings on various scientific and technical aspects of drug control and the high practical value of their outputs to national law enforcement and laboratory services, strongly recommended that such meetings and the publication of the technical manuals continue on a regular basis.

A consultative meeting comprised of fourteen experts was convened in December 1996 in Beijing by UNDCP's Laboratory Section in cooperation and with the Government of the People's Republic of China on the "Development of Guidelines for Validation of Analytical Methodology for Recommended Methods for Testing Drugs and Review of Methodology for the Identification and Analysis of Opiates in Seized Material". This manual reflects the conclusions of that meeting and provides practical assistance to national authorities by describing recommended methods to be used in drug testing laboratories for the identification and analysis of opium, morphine and heroin and may also serve as a guide in assessing methods in use in drug testing laboratories.

This manual is one in a series of similar publications dealing with the identification and analysis of various groups of drugs under international control. It combines and replaces previously published manuals on Recommended Methods for Testing Heroin (ST/NAR/6) and Recommended Methods for testing Opium and Crude Morphine (ST/NAR/11). Other manuals on Recommended Testing Methods include: ST/NAR/7 on Cocaine, ST/NAR/8 on Cannabis, ST/NAR/9 on Amphetamine and Methamphetamine, ST/NAR/12 on Illicit Ring-substituted Amphetamine Derivatives, ST/NAR/15 on Methaqualone/Mecloqualone, ST/NAR/16 on Benzodiazpine Derivatives under International Control, ST/NAR/17 on LSD, ST/NAR/18 on Barbiturate Derivatives under International Control, ST/NAR/19 on Peyote Cactus (mescal buttons)/Mescaline and Psilocybe Mushrooms/Psilocybin, ST/NAR/27 on Detection and Assay of Heroin and Cannabinoids, Cocaine, Amphetamine, Methamphetamine and Ring-substituted Amphetamine Derivatives in Biological Specimens, and ST/NAR/28 on Detection and Assay of Barbiturates and Benzodiazepines in Biological Specimens. These manuals can be requested from UNDCP's Laboratory Section (see address below).

The present and previous manuals suggest approaches that may assist drug analysts in the selection of methods appropriate to the sample under examination. The methods described therein are published in the scientific literature and have been used for a number of years in reputable laboratories. In identifying those methods, the Consultative meeting was aware that a number of other published methods in the forensic science literature also produce acceptable results.

USE OF THE MANUAL

In forensic drug analysis, the exhibits submitted for examination are very likely to show significant variation both in their physical/appearance form and chemical composition and not *all* methods described in this manual need apply to *all* samples suspected to consist of or contain opium, morphine or heroin. . The choice of the methodology and approach to their analysis remains within the discretion of the analyst and depends on the type of drug involved, the availability of reference materials and of appropriate instrumentation as well as on the level of legally acceptable proof in the jurisdiction within which the analyst works. Sophisticated methods are needed only for certain forensic requirements, such as comparison of samples or drug source determination.

In attempting to establish the identity of a controlled drug in suspected material, it is suggested that at least two independent analytical methods be used. For example, two uncorrelated TLC systems would count as two methods. Uncorrelated TLC systems in this context means either different solvent systems or different plate coatings. When possible, three entirely different analytical techniques should be used, for example: colour tests, chromatography (TLC, GLC or HPLC) and spectroscopy (IR or UV).

Attention is also drawn to the vital importance of the availability to drug analysts of reference books on drugs of abuse and analytical techniques. Moreover, the analyst must of necessity keep abreast of current trends in drug analysis, consistently following current analytical and forensic science literature.

UNDCP's Laboratory Section would welcome observations on the contents and usefulness of the present manual. Comments and suggestions may be addressed to:

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PRODUCTION OF ILLICIT OPIUM

The immediate precursor of heroin is morphine, and morphine is obtained from opium. Opium is the dried milky juice (latex) obtained from the unripe seed pods of *Papaver somnifer um L.*, more commonly referred to as the opium or oil poppy. Morphine has also been reported to be present in *Papaver setigerum*,[1] and as a minor alkaloid in *Papaver decaisnei*[2] and *Papaver rhoeas*.[3] However, there is no known instance of these poppies being used for opium production, and more recent work has cast considerable doubt as to the presence of morphine in *Papaver rhoeas*.[4] A major review by Kapoor on the botany and chemistry of the opium poppy is recommended additional reading.[5]

Opium latex is obtained from the seed capsule of the poppy[6] while the capsule is still in the green stage, usually seven or more days after flowering and petal fall. Physically, the opium latex is contained within laticiferous vessels which lie just beneath the epicarp of the seed capsule. The latex is harvested by making a series of shallow incisions through the epicarp which allows the latex to "bleed" onto the surface of the seed capsule. Most commonly, the latex is allowed to partially dry on the capsule surface, and is then removed by scraping the capsule with a specially designed hand tool. The dried latex is a malleable gum which is light to dark brown in colour, and is known as raw opium. The major constituents of raw opium are plant fragments, resins, sterols, triterpenoid alcohols, fatty acids, alcohols, polysaccharides, and more than thirty alkaloids.

Not all illicitly produced opium is used in the manufacture of heroin, as large quantities of raw opium are used for the production of "prepared opium." The principle use of prepared opium is for smoking. Also, there are considerable quantities of opium poppy grown for legitimate purposes. These uses include isolation of the opium alkaloids for medicinal purposes, extraction of the oils from the seed, and the use of the seed as food. For instance, a cold pressing of the poppy seed yields a white oil which has been used, in part, as a diluent in olive oil and as a flavoring agent in cooking. A second hot pressing of the seed gives an oil which has good drying properties and, in years past, was much used in oil paints and varnishes. Additionally, the use of the poppy seed as a condiment on bakery goods is very widely practiced, and the seed cake which is left after pressing can be used as animal food.

The opium poppy will grow in nearly any temperate region in the world, with the quantity of and timing of rainfall, average temperatures, and soil type being the major factors determining a suitable growing habitat. Proof for this is provided by the fact that legitimate opium poppy cultivation is known in Russia as far north as 55 degrees of latitude and in Argentina as far south as 40 degrees.[7] At this point in time, nearly all illicit opium production is located in southern Asia (South East and South West), South America, southern North America (Mexico) and Central America.

OPIUM ALKALOID CONTENT

The alkaloidal content of opium latex will vary substantially from plant to plant even within the same field. Hence, it is quite clear that agronomic variances affect alkaloidal composition. It is also widely accepted that varietal differences within *Papaver somniferum* can result in significant differences in alkaloidal composition. Nevertheless, in opium the same five alkaloids nearly always constitute the bulk of the alkaloidal fraction. Of these five major alkaloids, three are classified as phenanthrene alkaloids (morphine, codeine, and thebaine) and two as isoquinoline alkaloids (papaverine and noscapine). Table I lists the concentration ranges for these five alkaloids as determined for 1,414 raw opium samples of known origin. A sixth alkaloid, narceine, is frequently mentioned in the older literature as a major alkaloid in opium, but it most often occurs at a concentration range which is well below the other five alkaloids. Although all illicit opium growing regions are represented in Table I, they are not represented equally, since northwestern Thailand accounts for nearly 1,100 of the data sets. Nevertheless, the data does serve to describe minimum and maximum values for the five primary alkaloids. The majority of the data was acquired using High Performance Liquid Chromatography (HPLC: see Method B) while the remainder were acquired by Gas Chromatography (GC) using the method of Sperling.[8]

Analysis of noscapine by GC can be problematical, thebaine undergoes extensive rearrangement and decomposition in the GC injection port, and morphine requires derivatization prior to GC analysis. For these reasons, the use of GC methodologies is not preferred for quantification of opium alkaloids. However, in practice it has been found that when GC analyzed opium samples are re-analyzed by HPLC, the correlation between the data sets can be very good, even for these problem compounds, provided that sufficient care is taken to ensure optimum GC performance and complete derivatization of morphine.

TABLE I[9]

MAJOR ALKALOIDS FOUND IN RAW OPIUM			
alkaloids	**min%**	**avg%**	**max%**
MORPHINE	3.1	11.4	19.2
CODEINE	0.7	3.5	6.6
THEBAINE	0.2	3.1	10.6
PAPAVERINE	< 0.1	3.2	9.0
NOSCAPINE	1.4	8.1	15.8

OPIUM SOURCE DETERMINATION

Since the late 1940's, and on to the present time, there have been a large number of scientific investigations whose aim was to relate the relative and/or absolute alkaloid content of opium to the geographic source of the opium. Many of the earliest investigations were encouraged by the United Nations, and as a result a not insignificant body of work was published in the United Nations Bulletin of Narcotics during the 1950's.[10] These, and other later articles which followed, were optimistic about achieving that goal. However, to date there is no proven scientific method for determining the geographic origin of opium using alkaloid content. However, there are some consistent trends that are of note: (1) opium from Thailand and Myanmar (Laos may not be well represented in our data base) usually have the lowest papaverine content, (2) opium from Pakistan and Afghanistan tend to have the highest noscapine content, (3) the highest thebaine content is consistently found in opium from Iran, (4) opium obtained in the America's consistently have the lowest thebaine and highest papaverine levels, and (5) there is no statistically significant relationship between morphine or codeine content and opium origin. It is generally accepted that varietal differences do produce differing relative quantities of the opium alkaloids. However, the evidence gathered to date would suggest that agronomic and climatic differences could account for much of the noted trends.

OPIUM PREPARATIONS

Opium is generally encountered in one of four recognized forms: raw opium, prepared opium, opium dross, and medicinal opium.

In its purest form, raw opium is simply dried opium latex; however, it will always contain some quantity of plant fragments as a natural outcome of the harvesting methods employed, and can be cut with, among others things, flour, soil, rosin, or banana pulp. When fresh, it has a tar like consistency, is very sticky, and is usually a medium brown in colour. As raw opium ages, it will gradually become hard and brittle, and the colour will become darker, especially at the surface. Distinguishing features of raw opium are its characteristic odor, the presence of plant fragments, and the presence of meconic acid and porphyroxine.

Prepared opium (also known as cooked opium) is most often produced by dissolving raw opium in hot water, filtering to remove the insoluble materials, and evaporating until the filtrate again becomes a solid paste. This material is prepared almost exclusively for smoking purposes. Prepared opium, like raw opium, will give a positive colour test for meconic acid but obviously differs from raw opium in the absence or near absence of plant fragments. Additionally, a prepared opium will not give a positive colour test for porphyroxine, and the characteristic odor of raw opium is frequently absent.

Opium dross is the residue left after opium has been smoked. There are many local names for dross, for instance, in much of southeast Asia it is known as "chandu," while in Iran it is known as "sukhteh." Dross is both eaten and re-smoked after being added to prepared opium. The presence of dross in prepared opium is generally obvious as a charred material within the prepared opium, and a "burnt" odor is frequently present. Dross does not give a positive colour test for either meconic acid or porphyroxine.[11]

A medicinal opium is generally one of three preparations. The first is "granulated" or "powdered" opium (depending upon the final mesh size of the product), and is an opium which has been thoroughly dried at 70° C and diluted with lactose to give a morphine content between 10 and 10.5% (in Germany 9.8 to 10.2%) by weight. A second medicinal opium is known either

as "deodorized opium" or "denarcotized opium." This material is prepared by treating opium with petroleum ether, which removes both narcotine (noscapine) and the characteristic odor of opium. The concentration of morphine in denarcotized opium is also 10 to 10.5% by weight. The third medicinal opium has been modified rather extensively, and is commonly known within the U.S. under the trade name of Pantopon. This preparation is also known as "concentrated opium," Omnopon, or perhaps most commonly, Papaveretum. It is a mixture of morphine, codeine, papaverine, and noscapine as hydrochloride salts, with the morphine content adjusted to approximately 50% by weight.

ISOLATION OF MORPHINE FROM OPIUM

In 1803 Derosne[12] reported the isolation of a "salt of opium" from a potassium carbonate precipitation of an opium solution. Derosne is therefore frequently given credit for the first isolation of morphine, when in fact his isolate probably was not morphine. The credit for the first isolation of morphine may well belong to a French pharmacist by the name of Seguin. In 1804 Seguin presented to the *Academie des Sciences* the isolation of a substance which probably was morphine, but it was an additional ten years before his work was published.[13] Still others give first credit for the isolation of morphine to another pharmacist by the name of Sertürner; however, Sertürner himself believed that Derosne was the first to isolate morphine. Nevertheless, Sertürner did publish the isolation of morphine in 1805 , and undoubtably, Sertürner was the first person to grasp the chemical and biological significance of morphine.[14]

Of more importance in the world of clandestine manufacture are the publications which describe commercial processes for the extraction of morphine from opium. The best known of these publications describe the following processes: the Thiboumery and Mohr process, the Robertson-Gregory process, the Barbier process, the Heumann process, and the Schwyzer modification of the Robertson-Gregory process.[15] A discussion of the different morphine isolation techniques known to be utilized in clandestine laboratories is beyond the scope of this manual. However, the vast majority of these clandestine operations do follow the major tenets of the Thiboumery and Mohr process, the so called "lime method."

Morphine extraction by the lime method

Opium is dissolved in three times its weight of hot water and filtered. The residue is re-extracted and the two filtrates are combined.[a] The total filtrate volume is reduced to half and then poured into a solution of boiling calcium hydroxide.[b] Additional precipitants form, and they are captured by filtration. The captured residues are re-extracted with three parts of water, and again filtered to obtain a clean filtrate. The filtrates are combined, and the residues are removed from the process. The total combined filtrates are concentrated to a weight approximately twice that of the original opium, and the resulting solution is again filtered, with any captured residues being removed from the process. Then the solution is brought to a boil, and ammonium chloride is added; upon cooling, the solution is filtered to collect the precipitated morphine base. The morphine is then dissolved in a minimum volume of warm hydrochloric acid. As the solution

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a *The clandestine operator seldom separates the insoluble materials, rather they are carried through to the next step.*

b *Reduction of the solution volume is usually not performed in clandestine operations and lime is generally added to the filtrate, in solid form, immediately following the addition of opium to the hot water.*

cools, morphine hydrochloride precipitates, whereupon it is isolated by filtration.

In the field of clandestine opium extraction, there is great variance in the effort expended to produce a pure morphine, and several different purification methods are known to be employed. However, in general, it can be said that a product of high purity is often produced by those laboratories that produce morphine as the hydrochloride salt and/or where the morphine is the end product. In some cases, the product is near pharmaceutical quality. Conversely, in general, those laboratories that process from opium to heroin and produce the intermediate morphine as the free base, make a product that is often substantially less pure.

PRODUCTION OF HEROIN FROM MORPHINE

It was some seventy years after the first isolation of morphine from opium before the synthesis of diacetylmorphine was reported in 1874.[16] Commercial production by the Bayer Company, who named this new drug heroin, began in 1898. By the beginning of the twentieth century, heroin was widely accepted by the medical profession, and was typically used as a substitute for codeine and morphine in the treatment of tuberculosis and other respiratory diseases. It was also about this time that heroin first appeared in China. A few years after the 1925 International Convention on Narcotics, international controls began to limit the supply of heroin, and the clandestine manufacture of heroin began.

HEROIN SYNTHESIS

In nearly all the regions of the world, the synthesis of heroin begins with morphine isolated from opium. The synthesis of heroin is a simple one step acetylation reaction, and typically is performed by the addition of a large excess of acetic anhydride directly to morphine followed by heating the resulting solution to, or near, boiling. Generally, the final product is isolated by treating the cooled reaction mixture with sodium carbonate, and collecting the heroin base by filtration.

There are at least two regions in the world where clandestinely manufactured heroin is produced via non-traditional methods. One of these occurs in New Zealand, where what is known as the "Home Bake" process is practiced.[17] Home Bake involves the demethylation of codeine with pyridine hydrochloride[18] followed by acetylation of the resulting morphine using acetic anhydride. The second instance is known to occur in some areas of Russia and Poland, where an "acetylated opium" is produced from poppy straw.[19] Both of these procedures are thought to be exclusively used to produce small scale quantities for individual consumption.

In southwest Asia, heroin is most often sold as the free base without any further purification. In other parts of the world, the heroin is most often converted to the hydrochloride salt, and may undergo extensive purification procedures.

Variations in the acetylation process are many, but are usually limited to the use of differing solvents during and after the acetylation step, the use of reaction vessels of differing composition, the duration of the reaction, and the salt form of the morphine. In some instances, these variables are determined more by happenstance than by design.

Acetylating Agents

Acetic anhydride is by far the most common acetylating reagent used in the manufacture of heroin. However, on rare occasions, the use of two other acetylating reagents have been encountered, namely acetyl chloride and ethylidene diacetate (1,1-ethanediol diacetate).

In the case of ethylidene diacetate, it is not the actual acetylating agent. Rather, ethylidene diacetate is first decomposed to acetic anhydride by heating with catalytic amounts of either sulfuric acid or a zinc halide.

With somewhat greater frequency, small quantities of acetyl chloride, in conjunction with larger quantities of acetic anhydride, have been found at illicit heroin laboratories. In these instances, it is thought that a small quantity of the highly reactive acetyl chloride is being added to the acetic anhydride - morphine mixture as a reaction initiator. However, in the very recent past there have been indications that acetyl chloride is being utilized with increasing frequency in Southwest Asia. In some of these instances, it is thought that the acetyl chloride is being used to produce acetic anhydride by reaction with anhydrous sodium acetate.

In addition, in southwest Asia, there have been some recent suggestions that glacial acetic acid is being utilized for the acetylation of morphine. The esterification of simple alcohols using glacial acetic acid and an inorganic acid as catalyst is known, and has been used for large scale commercial productions.[20] The synthesis of 6-acetylmorphine via the reaction of morphine with glacial acetic acid was published by Wright[21] in 1874, and in 1984 Sy *et al*[22] duplicated the work of Wright. Neither produced heroin in significant yield, but in both cases 6-acetylmorphine was prepared in approximately 50% yield. Recently, using far stronger dehydrating agents than either of the previous works, an attempt was made to produce heroin from morphine using glacial acetic acid, with essentially identical results to these earlier works in that the primary product was 6-acetylmorphine and/or degradation products.[23] Although it is not beyond the realm of what is possible, it seems unlikely that glacial acetic acid will provide a suitable alternative to the classical choice of acetic anhydride for the acetylation of morphine.

Yields

A widely accepted yield estimate for the full process, opium-to-morphine-to-heroin, is as follows: 10 kg of opium produces approximately 1 kg of morphine base, which in turn will produce approximately 1 kg of heroin base. The morphine to heroin yield estimate is very reasonable; however, the opium to morphine yield must, at the very least, be considered optimistic.

ALKALOIDAL IMPURITIES IN HEROIN SAMPLES

In some high purity heroin samples, the presence of other alkaloids above 0.5% relative to heroin are limited to acetylcodeine and 6-acetylmorphine. Conversely, it is not uncommon for a heroin sample to contain a greater total quantity of alkaloidal impurities and byproducts than heroin. In those cases, noscapine is frequently the predominant alkaloidal impurity, but such heroin samples also contain considerable quantities of papaverine, acetylcodeine (occasionally a small quantity of codeine), and the two isomeric monoacetylmorphines; however, thebaine is almost never detected. In addition to meconin, small amounts of four synthetic by-products are occasionally detected, specifically: (1) acetylthebaol (principle decomposition product from the reaction of thebaine and acetic anhydride),[24] (2) 16,17-dehydroheroinium (although this compound is detected as a true synthesis impurity in some heroin samples, it is more typically observed as a GC injection port artifact),[25] (3) 3-[1-(1-carbomethoxy-ethyl)]-6-acetylmorphine (minor impurity observed when ethylidene diacetate is used for the acetylation of morphine),[26] and (4) 1-chloroheroin (observed when morphine is acetylated using contaminated acetyl chloride,[27] or when heroin is dissolved in certain chlorohydrocarbons which have been exposed to atmospheric oxygen).[28] Many other alkaloids are known to be present in opium, but they are

usually present at low levels relative to the morphine, and are almost never above the 0.5% level in heroin samples.

All heroin samples contain other neutral alkaloidal related impurities which are produced during the acetylation reaction. These impurities have either undergone loss of the amine ring system or have been converted to amides.[29] In either case, these compounds behave as neutrals, and are therefore easily separated from the bulk basic fraction. Once these compounds are isolated from the bulk of the sample, they can be analyzed with relative ease by GC. When expressed relative to heroin content, these compounds range in concentration from several parts per hundred for the most crudely refined heroin to less than an one part per hundred thousand for the most highly refined heroin. The GC/FID analysis of these neutral compounds has been successfully used to determine sample origin and for sample comparison.[30]

HEROIN SOURCE DETERMINATION

Each major geographic source area produces a heroin that, on average, is different from those found in the other producing regions. As a result, each major source area produces a heroin that can usually be recognized as a chemically distinct type. As noted in Table II below, there presently are four areas of the world which are generally recognized as being major sources of opium and heroin. In the past, southeast Asian opium and heroin production was generally considered to be more or less limited to the so called "Golden Triangle" area, which is principally comprised of the four common border areas of Thailand, Myanmar, China, and Laos. In recent years there has been a steady expansion of opium growing worldwide, and southeast Asia has been no exception. However, Thailand has been able to reverse the trend, and has reduced opium production quite significantly within its borders. Within southwest Asia, illicit opium production has become more concentrated in Afghanistan, while Mexican production is becoming more concentrated in western regions, and particularly in the southwest to include Guatemala. The fourth and last production area is found in South America, where at the present time opium production is largely limited to certain areas in northeastern Peru and along the slopes of the Andean mountain range in Colombia.

Southeast Asian Heroin

Southeast Asian (SEA) heroin is nearly always a white, sometimes slightly off-white powder. When uncut and present as the hydrochloride salt, SEA heroin samples will often have a particle size and appearance similar to laundry detergent, although most recently a finer and denser product is being encountered with increasing frequency. In most instances, uncut SEA heroin hydrochloride will be 80% or higher in purity, only rarely contains noscapine, and papaverine is nearly always absent. Heroin base from SEA is most often less pure than the corresponding hydrochloride, and there is an increase in the probability of higher codeine content, and an increased probability for the presence of noscapine at detectable levels.

Southwest Asian Heroin

Southwest Asian (SWA) heroin samples are far more variable than those from SEA. The most common form is a medium brown, free flowing, somewhat granular powder which looks much like its common U.S. street name, "brown sugar." Typically, when uncut, the heroin will be present as the free base at a purity of 40-60%; however, at this point in time there is some quantity being converted to the hydrochloride salt at sites outside of the source countries. The

remaining composition of these heroin samples are typically 20-30% noscapine, 2-6% papaverine, 5-9% acetylcodeine, plus many trace level alkaloid related impurities. The second most common SWA heroin is a tan to light brown, free flowing, somewhat granular powder which, when uncut, typically has a heroin purity of 60-85%, a noscapine content of 2-20%, a papaverine content between <1 to 4%, and an acetylcodeine content of 4-7%. These samples do not have a truly characteristic salt form, and have been encountered as the free base, the hydrochloride salt, the citrate salt, the tartrate salt, and any combination of these forms; however, they are most commonly found to be the hydrochloride. The final SWA heroin type is a free flowing white to off-white powder which is nearly always encountered as the hydrochloride salt. An uncut sample of this highly refined material can have a heroin purity >90%, with an acetylcodeine content between 2-5%.

Mexican Heroin

Mexican heroin is typically one of two types, which bear the street names of "black tar" and "Mexican brown." Black tar is, by a considerable margin, the most commonly encountered form of Mexican heroin within the U.S.A. A black tar heroin is dark brown to near black in colour, is a sticky amorphous tar-like substance, and will frequently have the characteristic odor of acetic acid. To reference a black tar heroin as being uncut (unadulterated) would be misleading, as virtually all black tar heroin samples contain significant quantities of tarry amorphous non-opium related materials. To date, few forensic laboratories have made an effort to identify these tarry amorphous diluent/s. Black tar heroin is known to be produced via several different methods. One method involves the addition of a sugar, such as lactose, into the acetylating mixture immediately after the acetylation of morphine has been completed. This process effectively kills the reaction, and almost instantly produces a tar-like product. It has been suggested that black tar heroin owes its popularity to the perception that it is difficult to adulterate. A typical "uncut" black tar will have a heroin purity of 30-60%, with acetylcodeine adding 1-6%, papaverine adding 0.5-3%, and noscapine adding an additional 1-4% to the total alkaloidal content. Not infrequently, papaverine will be more abundant than noscapine. A Mexican brown heroin is generally dark brown in colour and is a coarse granular powder. Frequently, a Mexican brown heroin is of lower purity than black tar heroin while other alkaloidal ratios are similar. This would certainly suggest a close relationship between these two sample types, but the details of that relationship have not yet been established.

South American Heroin

Uncut heroin from South America is nearly always a high purity (>90%), free flowing white powder. Typically, acetylcodeine will be less than 3.5%, averaging approximately 2.3%. When both of the positional isomers of acetylmorphine are summed, they seldom constitute more than 5% of the total sample, and 3-acetylmorphine will occasionally be at higher abundance than the 6-acetylmorphine isomer. Although this is not an unknown occurrence in heroin samples from elsewhere in the world, it is somewhat unusual, especially in the case of high purity heroin samples.

Table II was created using data compiled from 2,247 heroin samples of known origin. The data was acquired using the HPLC methodology described in *HPLC Method B*. The minimum, median, and maximum data shown in Table II were calculated by mathematical conversion of the various alkaloidal constituents in the heroin sample back to the fundamental opium alkaloids {i.e., expressed in terms of total morphine (M) as the anhydrous base where

M = (% Heroin x 285.34/369.42) + (% Acetylmorphine base x 285.34/327.38) + (% Morphine base)}. Similarly, "C" represents total anhydrous codeine base expressed in a similar manner to that used for the corresponding morphine data, while "P" and "N" represent papaverine and noscapine, respectively, both expressed as the anhydrous base.

TABLE II[31]

BASIC ALKALOID RATIOS FOR HEROIN SAMPLES					
alkaloidal ratio		**Southwest Asia**	**Southeast Asia**	**Mexico**	**South America**
C/M x 100	minimum	1.9	5.9	2.8	0.2
	median	6.6	12.6	5.3	2.2
	maximum	15.4	83.5	9.7	4.5
O6/M x 100	minimum	<0.1	0.2	0.3	0.1
	median	2.7	1.3	4.5	2.0
	maximum	46.0	47.5	51.3	50.3
P/M x 100	minimum	<0.1	<0.1	0.2	<0.1
	median	3.3	<0.1	2.3	0.2
	maximum	12.9	<0.1	5.6	13.2
N/M x 100	minimum	<0.1	<0.1	<0.1	<0.1
	median	40.2	<0.1	1.3	0.2
	maximum	303.4	2.6	149.0	11.2

Table II demonstrates that the median ratios are substantially different for noscapine, papaverine, and particularly codeine (each with respect to morphine) for heroin samples obtained from each of these primary source areas. Not surprisingly, the variance for each of these ratios are quite significant and there is a large overlap for each of the data sets across all four source regions. Hence, the data in Table II does not provide a fail safe method of identifying the source area of a given heroin sample; rather, Table II can only serve as an indication of source area. Given the foregoing, however, it is obvious that there are still instances when Table II can provide significant insight into the origin of a given heroin sample.

STRUCTURES AND PHYSICAL DATA FOR THE PRIMARY ALKALOIDAL CONSTITUENTS OF OPIUM AND HEROIN

This section provides physical information for the major alkaloidal constituents of opium and heroin samples. Data for each of the alkaloids provided is detailed under a commonly known name. The data includes other chemical, generic, and trade names which are more or less commonly encountered, the chemical structure, empirical formula, molecular weight (anhydrous base), and for the most common salt and hydrate forms, melting pc int and solubility data.[32] When standard material was available, and a conflict or absence of literature values was encountered, the melting point was determined via differential scanning calorimetry (DSC). A far more complete listing of chemical, common generic, and trade names is available in another United Nations publication.[33] Solubility data conform to the definitions as set forth by the "The National Formulary" and "The United States Pharmacopeia," and are detailed in Table III.

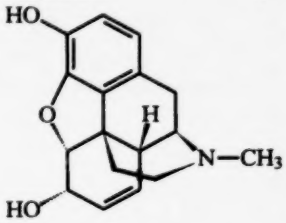
TABLE III

SOLUBILITY DEFINITIONS		
Description		Parts of Solvent required for 1 part of Solute
Very Soluble	(v sol)	Less than 1
Freely soluble	(f sol)	From 1 to 10
Soluble	(sol)	From 10 to 30
Sparingly soluble	(sp sol)	From 30 to 100
Slightly soluble	(sl sol)	From 100 to 1,000
Very slightly soluble	(v sl sol)	From 1,000 to 10,000
Practically insoluble or insoluble	(insol)	10,000 and greater

MAJOR CONSTITUENTS IN OPIUM AND CRUDE MORPHINE SAMPLES

Of the seven compounds which follow, six are truly major constituents in opium: morphine, codeine, thebaine, papaverine, noscapine, and meconic acid. The seventh compound, porphyroxine, is generally thought to be a minor component relative to the total alkaloid content of opium, although its content in opium is not well known. However, porphyroxine is a very important constituent, regardless of content, because of the central role it plays in the differentiation of raw opium from other opium preparations.

MORPHINE [Morphium, Morphia, Morphina, Duromorph, Nepenthe, Dolcontin]



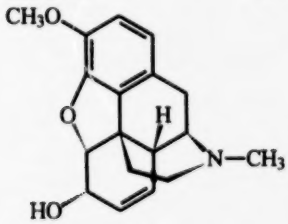
$C_{17}H_{19}NO_3$
MW = 285.34

Melting points (°C)

Base	Base ($1H_2O$)	HCl ($3H_2O$)	H_2SO_4 ($5H_2O$)
247-248(dec.)	255-257	200(dec.)	250(dec.)

Solubilities	Base	HCl	H_2SO_4
Water	v sl sol	sol	sol
Water, boiling	v sl sol	v sol	v sol(80°C)
Methanol	sol		
Methanol, boiling	f sol		
Ethanol	sp sol	sp sol	sl sol
Ethanol, boiling	sp sol	f sol(60° C)	sl sol(60° C)
Chloroform	v sl sol	insol	insol
Diethyl ether	v sl sol	insol	insol
Ethyl acetate	sl sol		
Benzene	v sl sol	insol	insol
Acetone	sl sol		

CODEINE [Methylmorphine, Morphine methyl ether, Codicept]



$C_{18}H_{21}NO_3$
MW = 299.37

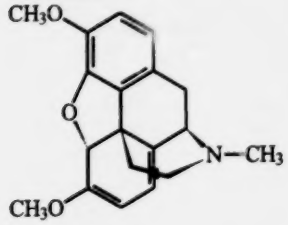
Melting points (°C)

Base ($1H_2O$)	HCl ($2H_2O$)	H_2SO_4 ($3H_2O$)
154-156	~280(dec.)	278(dec.)

Solubilities (gm/l)	Base	HCl	H_2SO_4
Water	sl sol	sol	sol
Water, boiling	sol	f sol	f sol
Methanol	f sol		
Ethanol	f sol	sp sol	v sl sol
Ethanol, boiling	v sol		
Chloroform	v sol	sl sol	insol
Diethyl ether	f sol		insol
Benzene	sol		
Acetone	f sol		

THEBAINE

[Paramorphine]



$C_{19}H_{21}NO_3$
MW = 311.38

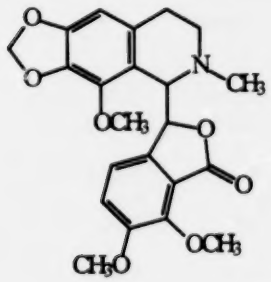
Melting points (°C)

	Base	*(Decomposes in dil. Mineral acids)*
	193	

Solubilities (gm/l)	Base	HCl
Water	v sl sol	sol
Ethanol	v sol	sol
Ethanol, boiling	v sol	
Chloroform	v sol	sol
Diethyl ether	sl sol	
Benzene	sol	

NOSCAPINE

[Narcotine, *l*-α-Narcotine, Narcosine, Methoxyhydrastine, Opian, Opianine]



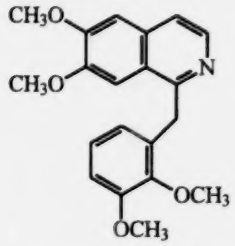
$C_{22}H_{23}NO_7$
MW = 413.43

Melting points (°C)

Base	HCl
176	220 (dec.)
	In general, salts are unstable

Solubilities	Base	HCl
Water	insol	f sol
Water, boiling	insol	sl sol
Ethanol	sl sol	f sol
Chloroform	f sol	f sol
Diethyl ether	sl sol	insol
Benzene	sol	
Acetone	sol	

PAPAVERINE [Papavérin]



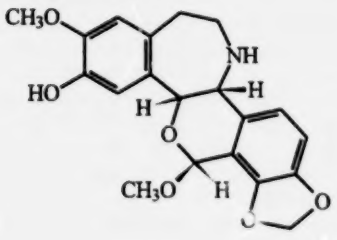
$C_{20}H_{21}NO_4$
MW = 339.38

Melting points (°C)

	Base	HCl ($1H_2O$)
	146-148	220-225(dec.)

Solubilities	Base	HCl
Water	insol	sp sol
Ethanol	sp sol	sol
Ethanol, boiling	f sol	
Chloroform	sp sol	f sol
Diethyl ether	sl sol	insol
Benzene	sol (hot)	
Acetone	sol	

PORPHYROXINE [Papaverubine D]



$C_{20}H_{21}NO_6$
MW = 371.39

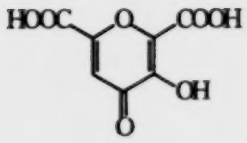
Melting points (°C)

Base
238-240

Solubilities	Base
Water	insol
Ethanol	sol
Chloroform	sol
Diethyl ether	sol

MECONIC ACID

[Oxychelidonic acid]



$C_7H_4O_7$
MW = 200.10

Melting points (°C)

	Free acid ($1H_2O$)
	120 (dec.)

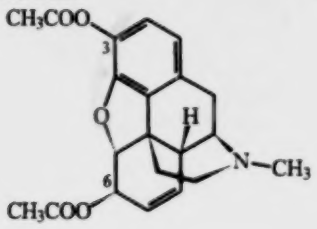
Solubilities

Water	sol	Water, boiling	f sol
Methanol	sp sol		
Ethanol	f sol		
Ethyl acetate	sp sol		
Benzene	f sol		

MAJOR CONSTITUENTS IN HEROIN SAMPLES

Except for rare occasions, a heroin sample will contain some detectable quantity of both 3- and 6-acetylmorphine, and acetylcodeine. Within a method designed for the analysis of heroin, the quantity of 3-acetylmorphine will frequently be at or near the method limit of detection, while 6-acetylmorphine will vary from a few tenths to 50% or more relative to heroin. The presence of 3-acetylmorphine in a heroin sample is generally attributable to incomplete acetylation of morphine, while the presence of 6-acetylmorphine is generally attributable to the hydrolysis of heroin. All other opium related alkaloidal impurities found in a heroin sample are due to their presence in the morphine prior to acetylation. Since papaverine does not contain a labile hydrogen and is quite stable, it remains unchanged by the acetylation process. As a result papaverine is present in the final heroin product at or near the same levels observed in the morphine prior to acetylation. However, noscapine is not stable to vigorous acetylation conditions, and if these conditions are prolonged, major quantities of the noscapine present in the morphine prior to acetylation can be converted to neutral products. Occasionally, codeine is detected in a heroin sample, while acetylcodeine is virtually always present at significant levels. In fact, like 6-acetylmorphine, acetylcodeine can be present at levels greater than 50% relative to heroin. In these cases, the analyst should be aware that a concurrent absence of papaverine and noscapine could indicate that the heroin was prepared from a morphine that was obtained via a codeine demethylation procedure.

HEROIN [Diamorphine, Diacetylmorphine, Acetomorphine]



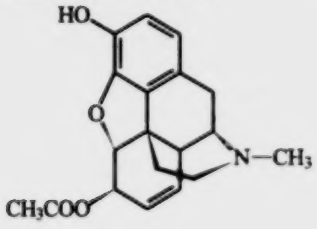
$C_{21}H_{23}NO_5$
MW = 369.40

Melting points (°C)

	Base	HCl ($1H_2O$)
	173	243-244

Solubilities	Base	HCl
Water	v sl sol	f sol
Water, boiling	dec	dec
Methanol	sol	f sol
Ethanol	sol	sol
Chloroform	f sol	f sol
Diethyl ether	sp sol	insol
Acetonitrile	sol	

6-ACETYLMORPHINE [O^6-Monoacetylmorphine, 6-O-Acetylmorphine]



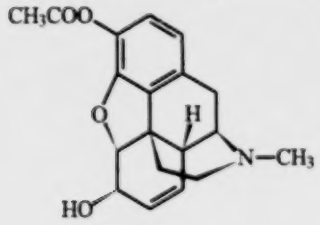
$C_{19}H_{21}NO_4$
MW = 327.37

Melting points (°C)

	Base	HCl ($3H_2O$)
	190 (DSC)	313 (DSC)
	200	265-267

Solubilities	Base	HCl
Water	v sl sol	sol
Methanol	sol	sol
Ethanol	sol	sol
Chloroform	sol	sol

3-ACETYLMORPHINE [O^3-Monoacetylmorphine, 3-O-Acetylmorphine]



$C_{19}H_{21}NO_4$
MW = 327.37

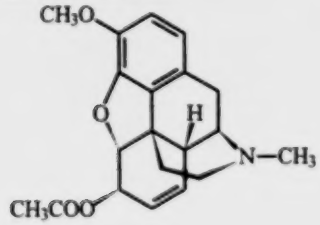
Melting points (°C)

Base	HCl
57-59	*Long term stability is questionable*

Solubilities

Base	HCl
no data available	

ACETYLCODEINE [6-acetylcodeine]



$C_{20}H_{23}NO_4$
MW = 341.41

Melting points (°C)

Base	HCl ($1H_2O$)
133 (DSC)	345 (dec.)
142 (sublimes)	

Solubilities	Base	HCl
Water	v sl sol	sol
Methanol	sol	sol
Ethanol	sol	sol
Chloroform	sol	sol

SAMPLING

The principle reason for a sampling procedure (plan) is to assure a correct and meaningful chemical analysis. Because most qualitative and quantitative methods for the examination of drugs require very small aliquots of material, it is vital that these small aliquots be representative of the bulk from which they have been drawn. Therefore, whenever possible, sampling should be carried out by a qualified analyst and should conform to the principles of analytical chemistry as set forth by national pharmacopoeas or in such publications as the "Official Methods of Analysis" which are produced by the International Association of Official Analytical Chemists (AOAC). A properly designed sampling procedure also produces an additional benefit in that it reduces the total number of analytical determinations required, thereby reducing the use of chemical and instrumental resources, and analyst time.

However, there are a few obvious guidelines which must supercede any sampling plan. First, in no case should selected samples from an exhibit be composited before the selected samples are "screened." Screening is the visual inspection of each sub-unit for differences in colour, markings, and other morphological properties, along with presumptive testing to detect similarities and/or differences between the individual units selected. Additionally, it may be necessary for the forensic analyst to discuss individual situations with the seizing officer and legal personnel to ensure adequate compliance with the needs of their respective law enforcement systems. For example, the analyst may need to preserve some part of an exhibit as visual evidence, or it may be necessary to perform separate assays on two items, rather than combining them prior to performing a single assay on the composite mixture. It is also important to remember that homogeneity of the sample is an issue, and in those events when the sample cannot be made homogeneous, a note stating this fact should be included in the analyst's final report.

Samples can be encountered in nearly any form one can imagine. Opium is usually encountered by the forensic analyst as a semi-dry, sticky, malleable gum, but it can also be encountered as the liquid latex or, if a medicinal product, as a free flowing powder. Similarly, heroin, which is usually encountered as a free flowing powder, can also be in liquid form or as a semi-dry, sticky, malleable gum. From the foregoing it is obvious that no single sampling plan will be applicable to all samples, and the analyst must be able to modify the sampling plan when necessary. However, all deviations from an approved sampling plan should be fully documented by the analyst.

SAMPLING OF A SINGLE PACKAGE

To illustrate some common sampling issues, it is appropriate to focus on the simplest sampling situation. This occurs when the submitted item consists of a single package of material. The first step upon receiving and opening an exhibit is to obtain a net weight. When practical, the material should be removed from its container or wrappings, placed into a previously weighed, substitute container, and the net weight of the material recorded. The next step is to ensure that the sample is thoroughly mixed (homogenous) before analysis. Some have suggested using materials made of plastic or glass (i.e., electrical insulators) as mixing containers. Although the use of a glass or plastic container as a mixing chamber can produce acceptable results, it can also result in static charge build up on the container walls during mixing, which would actually promote sample component separation rather than mixing. Hence, if an evidence independent mixing container must be used, a metal container, preferably stainless

steel, is a far wiser choice than most glass and, especially, plastic containers. Regardless of the methods employed, whenever possible the analyst should ensure that the sample is homogenous prior to analysis. It is recognized that, by their very nature, some samples are inhomogeneous, *e.g.*, raw opium, and that there is nothing the analyst can do to improve homogeneity without altering the nature of the sample. For these kinds of materials, if possible, the analyst should sample package units from multiple locations, preferably utilizing a coring technique, in order to obtain as nearly a representative sample as possible. It is also recognized that in the absence of a need to quantify an exhibit, the requirement for obtaining a homogenous sample may apply less rigorously.

A critical step in producing a homogenous sample from a package containing a powder is to ensure that the sample particle size is uniform. If the exhibit is large, some laboratories have found the technique of "coning and quartering" to be particularly useful. Coning and quartering is a procedure where, if necessary, a powder is reduced in particle size, then mixed, and placed onto a suitable flat surface to form a cone. The cone is flattened and divided into four equal quarters with opposing quarters being combined for the next step. This process is repeated using the selected quarters from the previous cone and quarter operation until the sample is reduced to a size suitable for analysis. If a powder contains large particles, it is most convenient to reduce particle size incrementally at each cone formation. Particle size reduction is generally sufficient when the entire sample will pass a 20 mesh screen. Although the mortar and pestle is an excellent tool for particle size reduction, it is unsuitable for large samples. For samples with particle sizes up to a few centimeters, an efficient method for sample particle size reduction is to place the material on a flat surface, and then work the powder material with the narrow edge of a thin metal bar using a vertical chopping motion. Such work is best performed in a well ventilated isolated area set aside for sample preparation. Personal safety make a dust mask, eye protection, gloves, and laboratory coat absolute necessities.

SAMPLING OF MULTIPLE PACKAGES

The analyst should visually examine both the packaging and the contents of the packages to ascertain if they appear identical. If visual examination indicates that the contents of some packages may be dissimilar, then the packages should be separated into as many groups as there are dissimilar contents, and each group should be individually sampled and analyzed as separate sub-exhibits. After samples have been taken from a suitable number of individual packages, and before the chosen samples are composited for analysis, each and every one of these samples should undergo presumptive testing. The composition of a sample determines the correct choice of the presumptive test. In many cases, a simple colour test is sufficient, while in other situations a more powerful technique such as TLC or GC may be required.

SAMPLING OF MULTIPLE PACKAGES FROM A SINGLE EXHIBIT BY THE SQUARE ROOT METHOD

The following sampling plan, the "square root" method, is accepted both by the AOAC and official drug compendia as a sampling tool, and therefore enjoys wide acceptance.

(a) If there are less than 10 packages - all packages should be sampled.

(b) If there are 10 - 100 packages - randomly select 10 packages.

(c) If there are more than 100 packages - randomly select a number of packages equal to the square root of the total number of packages rounded to the next highest integer.

SAMPLING OF MULTIPLE PACKAGES FROM A SINGLE EXHIBIT USING HYPERGEOMETRIC PROBABILITY DISTRIBUTION

An alternate approach for determining the number of samples taken from multiple package exhibits is offered by statistics in the form of the hypergeometric probability distribution.[34] Although experience has shown that the square root method produces reliable results, it does not provide a standard statistical foundation. However, a complication associated with the application of the hypergeometric probability distribution to sample selection is that the required calculations are cumbersome and are best carried out by computer. Hence, the following Tables are provided for the cases where all selected samples are positive for the suspected drug (Table IV), and where one or two of the selected samples were negative (Table V). When an analyst uses these sampling guidelines, and achieves the required number of positive results, there is a 95% probability that 90% of the exhibit contains the identified compound. In those cases where more than two negatives are encountered, the analyst should visually re-examine the exhibit, and attempt to segregate it into sub-exhibits which are uniform in content. Then each of the sub-exhibits can be sampled separately. In those, hopefully rare, instances when negative results exceed two, and the situation cannot be resolved by sub-dividing the exhibit, the analyst should consult his supervisor and/or exhaustively sample the exhibit.

TABLE IV

SAMPLING PLAN WHEN NO NEGATIVE RESULTS EXPECTED	
Total Number of Packages in Exhibit	**Number of Packages to be Selected**
10-12	9
13	10
14	11
15-16	12
17	13
18	14
19-24	15
25-26	16
27	17
28-35	18
36-37	29
38-46	20
47-48	21
49-58	22
59-77	23
78-88	24
89-118	25
119-178	26
179-298	27
299-1600	28
>1600	29

Use of Table V requires that the exhibit was previously sampled according to the guidelines set forth in Table IV, and that one or two of the samples taken gave negative results. The numbers specified in columns two and three of Table V are the number of positive results required per additional number of samples (positive / sample).

TABLE V

SAMPLING PLAN WHEN NEGATIVE RESULTS EXPECTED		
Total Number of Packages	**One Negative Test Result** *positive / sample*	**Two Negative Test Results** *positive / sample*
13 - 37	See Note Below	See Note Below Table
38 - 59	See Note Below	See Note Below Table
60 - 68	30 / 32	See Note Below Table
69 - 73	31 / 33	See Note Below Table
74 - 84	34 / 36	See Note Below Table
85 - 106	38 / 40	See Note Below Table
107 - 126	41 / 43	See Note Below Table
127 - 156	44 / 46	See Note Below Table
157 - 198	47 / 49	See Note Below Table
199 - 222	48 / 50	123 / 133
223 - 246	49 / 51	131 / 142
247 - 259	50 / 52	137 / 148
260 - 287	51 / 53	147 / 159
288 - 324	52 / 54	150 / 162
325 - 372	53 / 55	162 / 175
373 - 392	53 / 55	172 / 186
393 - 465	65 / 68	175 / 189
466 - 590	67 / 70	185 / 200
591 - 770	68 / 71	195 / 210
771 - 940	69 / 72	208 / 224
941 - 1150	69 / 72	221 / 238
1,151 - 1,500	70 / 73	234 / 252
1,501 - 10,000	72 / 75	245 / 264
10,001, - 30,000	72 / 75	255 / 274
>30,000	73 / 76	256 / 275

In these instances, additional packages should be screened until the total number of positive test results obtained in the first plus second sampling equals or surpasses 90% of the total number of packages in the exhibit.

ANALYSIS

It is difficult to distinguish between a positive colour test for morphine and one for heroin. Additionally, chromatographic systems that are suitable for the analysis of morphine/opium samples frequently need only to be modified slightly to also be suitable for the analysis of heroin samples. Finally, it is clear that the techniques utilized for the acquisition of spectral data for heroin and morphine differ only in minor detail. For these reasons, the following analytical sections will refer specifically to an opium, morphine or heroin analysis only when the issue under discussion applies exclusively to one or the other.

PROPERTIES OF OPIUM PREPARATIONS

Raw opium may be distinguished from other opium preparations by the presence of plant debris, meconic acid, and the trace level alkaloid porphyroxine. Prepared opium will contain meconic acid, most frequently some quantity of dross, and usually does not contain recognizable plant fragments or porphyroxine. Crude morphine can be distinguished from a prepared opium by the absence of plant fragments, porphyroxine, meconic acid, and usually the absence of the characteristic odor of opium. Presumptive colour tests for porphyroxine and meconic acid are given in the following section on colour tests.

MICROSCOPIC EXAMINATION OF OPIUM

Plant debris can be isolated for microscopic examination by exhaustively washing the opium with water. The residue will contain poppy capsule fragments and occasionally pollen grains. The poppy capsule fragments are epidermis composed of small 5 to 6 sided cells with strongly thickened walls and sometimes with stellate lumina; infrequent anomocytic stomata, approximately 17 µm wide by 25 µm long, or sometimes circular. Occasional sub-spherical pollen grains with 3 pores are also observed.[35]

COLOUR TESTS

Several different reagents are typically employed for colour and anion testing of opiates. Preparation of the reagents mentioned in this manual are described in annex II.

Typically, a colour test is the simplest and quickest chemical test that an analyst can apply to a sample. Most colour tests are quite sensitive; hence, only very small quantities of sample are necessary to complete a successful colour test. In fact, the colour test is best performed with the smallest of sample quantities, most often much less than 1 mg.

COLOUR TEST TECHNIQUES

Good analytical techniques are simply procedures which maximize the probability of a "true" result, and minimize the probability of a false positive. For colour tests, the most common source of a false positive colour test is a contaminated spot plate. Fortunately, a contaminated spot plate can be ruled out quite easily by first placing 1 to 3 drops of the reagent onto the spot plate, and then adding a small quantity of the sample to the reagent.

There are only three significant problems which an analyst may have with most colour tests and they are: (1) the obvious fact that they are not specific tests, (2) the use of too much sample, thereby overwhelming the chemical reagent (i.e., poor technique), and (3) contribution to the colour from other components in the sample. For the latter reason, opium, "black tar" heroin, and samples containing dyes can produce problematical colour test results, although in many instances a good positive colour test can be obtained even with these problem samples. For those cases when a colour test fails due to masking of the test colour, one of the following two procedures will frequently allow for an acceptable colour test:

(1) Place a small amount (approximately 10 mg) of the sample into a small test tube. Add approximately 10 drops of water, and stir the mixture with a glass rod in order to dissolve some of the sample. Make a small filter by tightly packing a very small quantity of glass wool into a disposable pipette. Draw the liquid up into a second disposable glass pipette, and place the liquid on top of the glass wool in the filter. With care the liquid can be filtered and deposited directly from the filter, one drop at a time to the edge of the colour reagent drop. Since the water will dilute the colour reagent, limit the amount of sample per spot on the spot plate to 1 drop, and use at least 3 drops of colour reagent. **Care must be taken to protect eyes and skin when using reagents made with concentrated sulfuric acid.**

(2) Treat the sample as in (1) except use approximately 1 ml of methanol (or 4:1 methanol-methylene chloride) instead of 10 drops of water. After filtering the methanol through glass wool, evaporate to dryness. Reconstitute the sample in a minimum amount of water, and then proceed with the colour test as in (1) starting at "one drop at a time."

The purpose of either of these procedures is to minimize colour contribution from sources other than those obtained by analyte reaction with colour reagent. Procedure (1) takes somewhat less time than (2) because of the lack of an evaporation step, while (2) is usually more successful in eliminating colour artifacts.

MECONIC ACID

Meconic acid is easily detected by a colour test using a 10% solution of ferric chloride. A small portion (approximately 1 mg) of the suspected material is placed on a spot plate. Add two drops of water, and with a glass rod, stir the sample until the water becomes brown. Transfer a drop to a spot plate well containing one or two drops of ferric chloride solution. A dirty blood red colour indicates the presence of meconic acid.

PORPHYROXINE

Porphyroxine is present at relatively low levels in raw opium, but its presence is easily confirmed by the intense red colour produced by the reaction of porphyroxine with mineral acids. The test involves placing a small quantity of opium on a spot plate, adding two drops of water, and mixing the sample until the water becomes brown. Transfer a drop to a spot plate well containing one drop of 2N hydrochloric acid. Upon gentle heating, a red colour develops if porphyroxine is present.

OTHER OPIATES

Table VI provides colour test results for the most common constituents of heroin and opium samples. The colours described in Table VI are the subjective judgement of an individual. Because of this subjective aspect, it is necessary for each analyst to test appropriate reference standards to assure that he or she can recognize each colour test result.

TABLE VI

Colour TEST RESULTS			
Alkaloid	**Marquis**	**Mecke**	**Frohde**
Heroin	purple violet	dark green	purple becoming grey/purple
Morphine	purple violet	dark green	purple becoming grey/purple
Codeine	purple violet	green/blue	blue/green
6-acetylmorphine	purple violet	dark green	yellow/green
Acetylcodeine	purple violet	dark green	purple becoming paler
Papaverine	no colour	dark blue	light green
Noscapine	bright yellow	green/blue	cherry red

The Marquis test is included in the test-kit supplied by the United Nations. The Marquis and other colour tests are also available from commercial vendors.

NOTES

When preformed properly, a negative colour test is generally quite reliable in establishing the absence of a target compound; however, positive results are only presumptive indications of the possible presence of a compound. Many other compounds, often harmless and uncontrolled by national legislation or international treaties, may give similar colours with a given colour test reagent. Therefore, it is mandatory for the analyst to confirm a positive colour test for any legally controlled compound by the use of an alternative technique.

ANION TESTS

From a forensic point of view, anion testing is applicable to all opiate samples with, of course, the exception of dross and raw or prepared opium. Morphine is commonly encountered as the hydrochloride salt, the sulphate salt, or as the free base, while on occasion it may be present as the tartrate salt. Heroin is most frequently encountered as either the free base or as the hydrochloride salt. It is not uncommon that the bulk of a heroin sample is the hydrochloride salt while a few percent (relative to the hydrochloride salt) is present as the free base. Another heroin salt which is encountered, but much less frequently, is the tartrate, and even less frequently a sample is found to be heroin citrate. On even more rare occasions, a heroin sample will be some combination of the hydrochloride, tartrate, and citrate salts, along with a small quantity of heroin base. Obviously, the salt form of a morphine or heroin sample cannot be ignored if accurate quantitative results are desired.

Anion testing for forensic purposes typically makes use of solubilities combined with selected reactions where results are determined by the presence or absence of a precipitant. There certainly are other methods available for the determination of anions, and some of these methods offer improved differentiation and identification for certain anions. However, these alternate techniques are, without exception, far more expensive and complicated to implement, and the results are sometimes less useful to the forensic scientist.

BASES

Heroin base is soluble in carbon tetrachloride, but all known salts of heroin are completely insoluble. Morphine base is insoluble in water, and has some slight solubility in benzene and chloroform.

HYDROCHLORIDE SALTS

Heroin hydrochloride is soluble in chloroform and methylene chloride. Heroin tartrate, heroin citrate, and most inorganic chlorides are insoluble in these solvents. Morphine salts are essentially insoluble in chloroform and benzene, while the base has some solubility in both solvents. When a water soluble chloride is treated with silver nitrate solution a white precipitate forms. The precipitate is insoluble in concentrated nitric acid, and after washing the precipitate with water, is soluble in dilute ammonia solution, from which it can be re-precipitated by the addition of nitric acid.

SULPHATE SALTS

Morphine sulphate is quite water soluble. When a water solution of a sulphate salt is treated with a solution of barium chloride a white precipitate forms, which is insoluble in hydrochloric acid.

TARTRATE SALTS

Heroin tartrate is insoluble in methylene chloride or chloroform, but is soluble in methanol. Morphine tartrate is water soluble while morphine acid tartrate is only slightly soluble in water. Silver nitrate will produce a white precipitate when mixed with a water solution containing either free tartaric acid or a tartrate salt. The precipitate is soluble in nitric acid. Congo red will produce a negative result with tartrate salts, but forms a blue colour when the free acid is present (congo red will also give a positive result if salicylic acid is present).

CITRATE SALTS

Heroin citrate is insoluble in methylene chloride or chloroform, but is soluble in methanol. Silver nitrate will produce a white precipitate when mixed with a water solution containing either free citric acid or a citrate salt. The precipitate is soluble in nitric acid. Acetic anhydride can be used to test for citrates and free citric acid. The test involves the addition of 0.5 ml acetic anhydride to a small amount of sample in a test tube, and heating the tube at 80°C for 10 minutes. A purple colour will develop if a citrate salt or free citric acid are present along with a tertiary amine, e.g., heroin.

THIN LAYER CHROMATOGRAPHY

Thin Layer chromatography (TLC) was invented nearly fifty years ago. In the original published work a list of the advantages of the technique were given,[36] and nearly every point on that list of advantages are realized by the analyst of today. It is rapid (an analysis is rarely longer than thirty minutes), sensitive (sub-milligram quantities of analyte required), amenable to a wide variety of substances and visualization techniques, and is inexpensive.

TLC PLATES

Whether obtained commercially or prepared by the analyst, TLC plates should be coated with activated silica gel G at a thickness of 0.25 mm. If the analyst desires, silica gel containing an additive which fluoresces under UV light may also be used. TLC plates are commercially available in a variety of sizes. The actual size used depends upon the number of samples to be simultaneously developed, and the size of the TLC tank. An optimum run is considered to be approximately 10 cm; therefore, the use of plates which provide a minimum of 9 cm of development is recommended. Typical plate sizes are 20 x 20 cm, 20 x 10 cm, and 10 x 5 cm where the 10 x 5 cm plate should be used with the 10 cm side vertical in the TLC tank.

TLC TANK

The TLC tank and lid should be clear glass and the tank should be lined with adsorbent paper to assist in saturating the tank volume with solvent vapors. The lid should fit tightly to minimize solvent loss from evaporation. A glass tank rim which has been ground or has been coated with a thin film of petroleum jelly provides an adequate seal. The developing solvent in the TLC tank should be between 0.3 and 0.5 cm in depth.

SPOTTING

The starting point of the run, i.e., the "spotting line," should be 1 cm from the bottom of the plate. The spacing between applications of sample (spotting points) should never be less than 0.8 cm apart, and in no case should a spot be placed closer than 1.5 cm to the side edge of the plate. The size of the sample spot should be as small as possible ($\leq$ 2 mm); otherwise, diffuse spots will be produced during development. To achieve a small diameter spot, it may be necessary to apply the sample solution in several small aliquots rather than by a single discharge. The spots may be dried by cold or hot air between applications. If hot air is used, care must be taken to ensure that no component of the mixture under investigation is thermally labile.

DEVELOPING SOLVENT

The developing solvent should be made as accurately as possible by use of pipettes and measuring cylinders. If the same solvent systems are used daily, it may be convenient to obtain each component via an automatic dispenser. Mixing of solvents may be accomplished within the TLC tank. The developing solvent must be in the TLC tank for a time sufficient to allow saturation to be achieved. With paper lined tanks, this takes approximately 5 minutes. It is important to note that for the developing systems A and B (Table VII), the solvent must be renewed after a maximum of 3 runs, or ideally, after each development.

DEVELOPMENT LINE

A 20 cm tall TLC plate should have a "development line" scratched into the coating 11 cm from the bottom, i.e. the "spotting end," while for a 10 cm plate, the end of the plate is the development line. An analysis should be terminated when the solvent migrates to the development line. It is imperative that the analyst monitors the progress of solvent up the TLC plate. The plate must be removed from the development tank as soon as the solvent reaches the "development line"; otherwise, diffuse spots will result.

PREPARATION OF SAMPLES AND STANDARD SOLUTIONS

Standard Solutions

Standard solutions of heroin, morphine, codeine, 6-acetylcodeine, 6-acetylmorphine, papaverine, and noscapine should be prepared at a concentration of 1 mg/ml in methanol. All standards may be combined into a single standard solution; however, two solutions are recommended: 1) heroin, morphine, noscapine, papaverine, and 2) codeine, 6-acetylmorphine, acetylcodeine. Irrespective of salt form, the compounds always move on the TLC plate as the free base; therefore, the standard solutions can be prepared using either a salt or the free base.

although the salts, with the exception of noscapine, are generally somewhat more stable in solution. Spot 5 µl of the standard solution(s) to the TLC plate.

Sample Preparation

The following extraction technique is suitable for the isolation of the heroin, opium, and crude morphine from seized materials. Dissolve 5 mg of sample for each 1 ml of methanol, and place both a 1 µl and a 5 µl spot onto the plate.

TABLE VII[37]

RECOMMENDED SOLVENT SYSTEMS					
Solvent System A		Solvent System B		Solvent System C	
Toluene	45	Ethyl Acetate	85	Methanol	100
Acetone	45	Methanol	10	Conc. ammonia	1.5
Ethanol	7	Conc. ammonia	5		
Conc. ammonia	3				

Visualization

Plates must be dry prior to visualization. Drying can be accomplished at room temperature, or more quickly in an oven set no higher than 120°C, or by use of a hot air blower. It is important for proper colour development that all traces of ammonia be removed from the plate. The following visualization methods are recommended:

1.) UV light, usually at 254 nm if there is a fluorescent additive in the silica gel.
2.) Dragendorff's spray reagent (see annex II for preparation) gives orange spots on yellow background with opium alkaloids.
3.) Acidified potassium iodoplatinate spray reagent (see annex II for preparation) gives blue to purple spots with the opium alkaloids.

TABLE VIII[38]

RESULTS			
	R_f values		
SOLVENT SYSTEMS	A	B	C
HEROIN	57	49	47
MORPHINE	19	20	37
CODEINE	40	35	33
PAPAVERINE	72	69	61
NOSCAPINE	88	78	64
6-ACETYLMORPHINE	53	44	46
ACETYLCODEINE	69	54	44

NOTES

R_f values shown above are for general reference only. Small changes in TLC plate composition and solvent systems can result in significant changes in the R_f values. Hence, the analyst must only compare data with those obtained against suitable reference standards applied to the same TLC plate as the sample.

GAS CHROMATOGRAPHY

The first instrument-based chromatographic system, the gas chromatograph (GC), was developed some forty years ago. This early system was limited to compounds within a very narrow vapor pressure range, where because of peak broadening most separations were limited to thirty minutes or less. Generally when using such a system, no more than six or eight compounds could be baseline resolved, and at that time this was considered to be high art. Today a state of the art micro-bore capillary column GC systems can perform baseline separations between a hundred or more compounds, and usually do so with run-times much less than thirty minutes. However, due to hardware limitations, such a state of the art system is not yet an instrument that is suitable for routine analytical use. Rather, today the GC instrument of choice for routine analytical work is the narrow bore capillary GC. The narrow bore capillary GC provides a level of performance more similar to a micro-bore GC system than that of a packed column system, while it is easier to use and to maintain than a packed column system. Because of their high resolving power and thermal stability, the narrow bore capillary column allows for the direct determination of both major and minor constituents of opium, and morphine samples without the need for a prior extraction step.

It is recognized that there are laboratories, which for a variety of reasons may wish to maintain a packed column system. For those laboratories two methods are described for the analysis of heroin, morphine, and opium. Additionally, a GC procedure utilizing a megabore capillary column is also described since many GC systems that are designed for packed columns can be converted to use megabore columns.

The ability of a GC column to separate a large number of components (i.e., resolving power) is directly related to column efficiency, which is generally expressed in theoretical plates/meter. A packed column will generally have efficiencies in the range of 200 plates/m while the megabore capillary column will be closer to 1,500 plates/m, and the narrow bore capillary column will be in the range of 4,000 plates/m. However, while the resolving power of the capillary column becomes significantly greater as one proceeds from packed to megabore to narrow bore there is also a corresponding decrease in the amount of sample which can be loaded on the column. Column loading capacity becomes an important consideration when the sample being analyzed has very large differences in component quantities. Estimating the on-column quantity of analyte which will overload a given column can be difficult. However, it is useful to have some knowledge of these limits; therefore, the following estimates are offered as approximate guidelines: 4 mm bore packed column, ~200 µg; 2 µm film thickness 0.53 mm megabore, ~2 µg; 0.25 µm film thickness 0.25 mm narrow bore, 50-75 ng).

PACKED COLUMN METHODS

METHOD: A

Operating Conditions

Detector:	FID
Column:	6 ft (or 2 m) x 2 to 4 mm I.D.
Packing:	3% Dimethyl Silicone (SE-30 or OV-1)
Carrier Gas:	N_2 at 70 ml/min.
Injection size:	2 to 5 µl as appropriate
Temperatures:	Injector: 275 °C Detector: 275 °C Oven: 230 °C
Internal Standard:	Docosane or other suitable *n*-alkane (100 mg/5.0 ml methanol)
Derivatization Reagent:	N,O-bis-trimethylsilylacetamide (BSA)

PREPARATION OF SAMPLES AND STANDARD SOLUTIONS

Standard solutions for heroin, 6-acetylmorphine, and acetylcodeine

A standard solution of heroin hydrochloride is prepared by accurately weighing 25-50 mg into a 50 ml volumetric flask. The heroin hydrochloride is dissolved in a minimum volume of methanol followed by the addition of 1 ml of internal standard solution. The solution is then made up to a final volume of 50 ml with chloroform. A standard solution of 6-acetylmorphine and acetylcodeine is prepared by accurately weighing 25-50 mg of each into a 50 ml volumetric flask. The standards are dissolved in a minimum volume of methanol followed by the addition of 1 ml of internal standard solution. The solution is then made up to a final volume of 50 ml with chloroform.

Heroin sample preparation

Treat a heroin sample in the same fashion as the heroin standard (using at least 25 mg of sample) to give a final heroin concentration between 0.5 and 1.0 mg/ml.

Heroin derivatization

To 0.5 ml of the standard solution add 0.5 ml of BSA in a stoppered vial, and heat at 100 °C for 10 minutes. Treat 0.5 ml of the heroin sample solution in the same manner.

Opium sample preparation

For most forensic purposes, only morphine needs to be quantified. The following sample procedure removes meconic acid from the sample matrix, and is recommended for the quantitative determination of morphine. An accurately weighed opium sample of approximately 300 mg is placed into a small beaker. Add a 2 ml aliquot of the internal standard solution to the beaker and, with stirring, slowly add 10 ml of a 2.5% acetic acid solution. When the sample has completely dispersed, transfer the mixture to a separatory funnel, wash the beaker with 10 ml of water, and add the wash to the separatory funnel. Add

5 gm of sodium chloride, and then add dilute ammonia solution until the pH is approximately 10. Extract with seven - 40 ml portions of chloroform-isopropanol (3:1). To the combined organic extracts add a sufficient amount (approximately 10 gm) of anhydrous sodium sulfate to ensure that a clear solution is obtained. Shake the mixture, filter and evaporate the filtrate to dryness, if possible, under reduced pressure in a rotary evaporator. Dissolve the residue as completely as possible in 20 ml of methanol, and place 1.0 ml in a small stoppered tube. Remove the solvent as before, and dry the residue thoroughly.

Opium and morphine standard solutions

A standard morphine stock solution is prepared by dissolving 100 mg of morphine hydrochloride standard in methanol to give 5 ml of solution. A 1.0 ml aliquot of the morphine hydrochloride stock solution plus a 1.0 ml aliquot of the internal standard solution are diluted with methanol to a final volume of 10.0 ml. Place 1.0 ml of this standard working solution in a stoppered test tube and complete the procedure described above for the preparation of the opium sample starting with the words "Remove the solvent."

Morphine sample preparation

The purpose of the extraction steps described above was to purify morphine and remove meconic acid. Illicit morphine does not normally contain meconic acid. Therefore, illicit morphine samples may be prepared using the procedure described above for the standard morphine hydrochloride solution.

Derivatization of opium or morphine samples

To the dry residue add 1.0 ml of BSA. Insert the stopper in the tube, shake gently, and allow the tube to stand at room temperature for 10 minutes before injecting 1 µl into the gas chromatographic column.

Elution order: Codeine, Acetylcodeine, Morphine, 6-Acetylmorphine, Heroin, Papaverine. Noscapine does not usually elute under the conditions of this method.

METHOD: B[39]

Operating Conditions

Detector:	FID
Column:	2 m x 2 mm I.D.
Packing:	2.5% Methylphenyl Silicone 5% Phenyl (SE-52)
Carrier Gas:	N_2 at 30 ml/min.
Injection size:	1 µl
Temperatures:	Injector: 270 °C
	Detector: 300 °C
	Temperature programme:
	1.) 240 °C, isothermal for 2 min.
	2.) 24 °C/min to 280 °C
	3.) Hold isothermal for 6 min.

4.) End of programme

Internal Standard: Amitriptyline (preparation is described below)

Derivatization Reagent: Propionic Anhydride

PREPARATION OF SAMPLES AND STANDARD SOLUTIONS

Internal Standard preparation

Dissolve 100 mg of amitriptyline hydrochloride in a few millileters of water; after the addition of a few drops of concentrated ammonia solution, the solution is extracted three times with ethyl acetate. The combined extracts are dried over anhydrous sodium sulfate and filtered into a 100 ml volumetric flask. Propionic anhydride (10 gm) is added and the volume increased to 100 ml with ethyl acetate.

Sample and standard solutions preparation

The equivalent of 50 mg of heroin or morphine, or 200 mg of opium, is accurately weighed into a 15 ml screw-capped tube to which 2 ml of water is added. Five milliliters of the internal standard mixture and 1-2 gm of sodium hydrogen carbonate (sodium bicarbonate) or di-sodium hydrogen phosphate are added to the tube, and after capping, the tube is mixed on a Vortex mixer for 10-15 minutes (if sodium bicarbonate is used, the analyst should ensure that the evolution of carbon dioxide has completely ceased before capping- the use of di-sodium hydrogen phosphate circumvents this potential problem). After mixing, centrifugation will help in separation of the aqueous and organic phases. After centrifugation, the organic layer will be on top of the aqueous, and is easily sampled directly from the tube for injection into the GC column.

Elution order: Amitriptyline, codeine, acetylcodeine, morphine, heroin, 6-acetylmorphine, papaverine. Noscapine does not usually elute under the conditions of this method and the degradation products of thebaine elute after acetylcodeine.

NOTES FOR PACKED COLUMNS

Prior to use, all packed columns must be conditioned. Usually the conditioning temperature should be at least 30 °C above the maximum temperature used in the analysis. However, if this temperature exceeds the maximum temperature limit specified by the manufacturer, then a smaller temperature differential must be used, and the conditioning period substantially extended. Typically, columns are conditioned overnight for a minimum of 15 hours. It is imperative that conditioning be carried out with a leak free injection system, with normal carrier gas flow, and with the column disconnected from the detector.

The analyst is advised to silanize glass columns frequently to avoid adsorption of morphine during GC determinations, and maintain a leak free system.

CAPILLARY COLUMN METHODS

MEGABORE CAPILLARY COLUMN

Operating Conditions

Detector:	FID at 22 ml/min H_2 and 350 ml/min air
Column:	Fused silica, 10 m x 0.53 mm I.D., 2 µm crosslinked 50% Phenyl and 50% Methyl Siloxane stationary phase (HP-17)
Carrier Gas:	H_2 at approximately 8 ml/min. at 250 °C oven temperature
Injection technique:	Splitless, 1 µl
Make-up gas:	Ar or N_2 at 30 ml/min.
Temperatures:	Injector: 230 °C Detector: 300 °C Temperature programme: 1.) 250 °C, with 0 min hold time. 2.) 2 °C/min to 280 °C 3.) End of programme
Internal Standard:	3 gm of Benzopinacolone is dissolved into 1:1 isopropanol/2,2-dichloro-ethane, and made up to 2 liters.

PREPARATION OF SAMPLE AND STANDARD SOLUTIONS

Heroin Standard & Sample Solutions

An amount of sample equivalent to 5-10 mg of heroin (depending on the purity of sample) is accurately weighed into a 20 ml vial, and then 10 ml of internal standard solution is added. The solution is sonicated for 25 minutes and allowed to settle. If the solution is turbid, it can be filtered by a syringe coupled with a disposable 0.2 µm nylon membrane filter.

Elution order: Codeine, morphine, acetylcodeine, 6-acetylmorphine, heroin, benzopinacolone, papaverine, noscapine.

NOTE

This GC quantitative method is capable of dealing with samples adulterated with caffeine, theophylline, chlorpheniramine, and carbetapentane. Analysis of heroin samples having any other additive need to be studied by the user.

NARROW BORE CAPILLARY COLUMN[40]

Operating Conditions

Detector:	FID at 30 ml/min H_2 and 400 ml/min air
Column:	Fused silica, 30 m x 0.32 mm I.D., 0.25 µm chemically bonded Dimethylpolysiloxane stationary phase (HP-1)
Carrier Gas:	He at 61 cm/sec measured at 150 °C oven temperature
Injection technique:	Split (ratio 1:15), 1 µL
Septum purge:	2 ml/min
Make-up gas:	Ar or N_2 at 25 ml/min
Temperatures	Injector: 250 °C Detector: 310 °C Temperature programme: 1.) 150 °C, with 0 min. hold time. 2.) 9 °C/min to 300 °C 3.) Isothermal for 2.4 min. (Heroin and Morphine) or for 5.4 min (Opium) 4.) End of programme
Internal Standard:	*n*-Tetracosane
Derivatization Reagent:	N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA)

PREPARATION OF SAMPLES AND STANDARD SOLUTIONS

Heroin Sample & Standard Solutions

For the quantitation of heroin, and the detection of other constituents in heroin samples, accurately weigh an amount of a heroin sample equal to approximately 5 mg of heroin and 1 mg of *n*-tetracosane into a small test tube or injection vial. Add 1 ml of chloroform, 200 µl of pyridine, and 150 µl of MSTFA to the tube, and then heat the mixture for 10 minutes at 70 °C. Remove from heat, and let stand at room temperature for 1 hour. Prepare a standard heroin solution in the same manner.

Opium, Morphine Sample & Standard Solutions

For the quantitation of morphine, and the detection of other constituents in opium or morphine samples, accurately weigh about 5 mg of illicit morphine, or 10 mg of opium, and 1 mg of *n*-tetracosane into a small test tube or injection vial. After adding 1 ml of chloroform, 200 µL of pyridine, and 150 µl of MSTFA, the mixture is heated for 10 minutes at 70 °C. Let stand at room temperature for 1 hour for morphine or overnight for opium samples. Prepare a morphine standard solution in the same manner.

Elution order: *n*-Tetracosane, codeine, acetylcodeine, morphine, 6-acetylmorphine, heroin, laudanosine, papaverine, cryptopine, noscapine.

NOTE

This GC method can easily separate most of the common adulterants and diluents present in illicit samples. However, if diazepam, quinine, or phenylbutazone are present, a change of the chromatographic conditions is required because of overlap with the internal standard or the heroin peak.

GENERAL NOTES ON GC

If routine operation of the GC involves temperature programing with large temperature changes (+100 °C), the analyst should be aware that column fittings will require frequent attention.

Ensuring that the injection needle is well formed and sharp will assist greatly in maintaining a leak free system, but even so, the septum should be changed frequently (i.e. before a leak develops.)

When changing a septum, the minimization of oxygen intrusion into the column will greatly prolong the useful life of the system. Septa should be changed with column and injection port at or below 180 °C, and as quickly as possible in order to minimize oxygen intrusion. If using a packed column or an injection port liner containing packing material, the column flow must be turned off, and the column pressure reduced so that the packing material is not disturbed when the septum is removed. Finally, after changing a septum, the column should be purged at the full flow rate for at least 10 minutes before re-establishing the injection port and column at higher temperatures.

Injection port and detector should be regularly in order so as to avoid decomposition of samples and loss of detector sensitivity. It is important to remember that the physical conditions within a GC injection port (i.e., high temperature and high pressure) can frequently cause and/or enhance unwanted reactions. These reactions are most often either thermally-induced elimination or esterification reactions, and they are either enhanced or catalyzed by contaminants in a dirty injection port. Some specific problems associated with the analysis of heroin samples are the elimination of the 3-acetyl group from heroin in the GC injection port to give 6-acetylmorphine, and the so-called *trans*-esterification reaction. *Trans*-esterification reactions are most troublesome when acetylsalicylic acid is co-injected with morphine or codeine.[41] Additionally, the use of an alcohol as the injection solvent can also result in esterification of certain analytes; however, esterification reactions are usually not a significant problem if the injection port is properly maintained.

Silylating reagents must be handled with care, as they are very reactive and sensitive to moisture.

For an alternative GC method which does not use derivatization, see the work of Gough and Baker.[42]

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The requirement for the analyte to be in the gas phase has always been recognized as a severe limitation to the universal application of GC, and was a major impetus in the development of HPLC, where the physical limitation is that the analyte must have some solubility in a liquid. When using a GC there are some suitably volatile analytes which undergo thermally induced reactions that make qualitative and quantitative assessments difficult or impossible. However, there is an analogous problem with HPLC since most methods employ a mobile phase which is either acidic or basic in character, and there exists a corresponding set of analytes which are incompatible with these conditions. Therefore, it would be inappropriate to suggest that either of these techniques is preferable for all analytes of forensic interest. Nevertheless, HPLC is probably the method of choice for the quantification of opium and morphine samples.

METHOD A — Opium and Morphine

Operating Conditions

Detector:	UV at 280 nm	
Column:	Octadecyl-silica, 300 x 3.9 mm I.D. (μBondapak C_{18} or equivalent)	
Mobile Phase:	Water	40
	Acetonitrile	60
	Triethylamine	0.1
Flow rate:	2 ml/min.	
Injection Volume:	5-20 μl	
Internal Standard:	Accurately weigh 10 mg of morphine (base or hydrochloride) and dissolve it in 10 ml of mobile phase.	

PREPARATION OF SAMPLES AND STANDARD SOLUTIONS

Opium

Disperse 100 mg of opium with 10 ml of saturated aqueous sodium chloride at pH 10 (adjust with dilute ammonia). Extract the mixture using three 20 ml portions of chloroform/isopropanol (3:1). Filter the organic layers through anhydrous sodium sulfate, or phase separation paper, and evaporate the organic solvent to dryness. Take the residue up in 10.0 ml of mobile phase.

Morphine

A morphine sample is prepared in the same manner as the morphine internal standard solution. Accurately weigh approximately 10 mg of morphine sample and dissolve in 10.0 ml of mobile phase.

Elution order: Morphine, codeine, thebaine, noscapine, papaverine.

NOTES

This system does not resolve morphine and meconic acid; hence, a prior extraction is required for the determination of morphine in opium. A morphine sample, however, may be analyzed by direct dissolution of the sample in mobile phase. This method is recommended for the quantification of morphine only, as separation between the other alkaloids is not always adequate. Using an isocratic mobile phase flow conditions, a typical analysis is completed in less than 5 minutes. The short run time allows for high sample throughput.

HPLC METHOD B[43] Heroin, Related Alkaloids, and Adulterants

Operating Conditions

Detector: UV Diode Array, monitor 3 wavelengths: 210, 228, and 240 nm

Column: Partisil 5, ODS 3, 125 mm x 3.2 mm I.D.

Mobile Phase: (1) Phosphate buffer. Using HPLC grade water, make up a solution of 3% 2N Sodium Hydroxide and 1% Phosphoric Acid, with 3 to 4.5 ml Hexylamine added per 870 ml of total buffer volume.

(2) Methanol

Injection Solvent: Injection solvent is prepared as a 89:10:1 ratio of HPLC grade water, acetonitrile and glacial acetic acid, and is adjusted to pH 3.7 using 2N sodium hydroxide.

Flow rate: 0.76 ml/min

Injection Volume: 20 µl

Programmed Gradient:

	Time (min)	Methanol %	Buffer %
Equilibrate	15	5	95
1	20	30	70
2	6	30	70
3	10	80	20
4	4	80	20
5	5	5	95
6	End of Programme		

Internal Standard: Propiophenone (heroin) or Procaine HCl (opium/morphine)

PREPARATION OF SAMPLES AND STANDARD SOLUTIONS

Heroin Internal Standard Solution

A stock solution is prepared by accurately weighing 40 mg of propiophenone into a 100 ml volumetric flask and diluting to volume with injection solvent.

Opium Internal Standard Solution

A stock solution is prepared by accurately weighing 50 mg of procaine HCl into a 100 ml volumetric flask and diluting to volume with 9:1 phosphate buffer-methanol.

Heroin Standard Solution

A stock solution is prepared by accurately weighing approximately 90 mg heroin base, 1.5 mg of morphine base, 2.0 mg of codeine base, 1.5 mg of 6-acetylmorphine base, 3.4 mg of acetylcodeine base, 1.0 mg of noscapine base, and 1.0 mg of papaverine base into a 100 ml volumetric flask, followed by the addition of 10 ml of Internal Standard solution. The mixture is diluted to volume with injection solvent and is stored in the cold until ready for use.

Opium Standard Solution

A stock solution is prepared by accurately weighing 5.0 mg of morphine base, 2.0 mg of codeine base, 2.0 mg of thebaine base, 3.0 mg of noscapine base, and 2 mg of papaverine base into a 100 ml volumetric flask. Then add 10 ml of internal standard solution, and dilute to volume with 9:1 phosphate buffer-methanol.

Heroin Sample Solution

Accurately weigh sample into a 100 ml volumetric flask to give an approximate heroin concentration of 0.9 mg/ml. Add 10 ml of the internal standard solution to the flask, and dilute to volume with injection solvent. Sonicate to complete solvation.

Opium Sample Solution

Approximately 40 mg of raw opium is weighed into a 100 ml volumetric flask. Add 10 ml of methanol and reflux the solution for 30 minutes. After cooling, 10 ml of internal standard solution are added, and the mixture is brought to volume with phosphate buffer.

Heroin sample elution order:	Morphine, codeine, 3-acetylmorphine, 6-acetylmorphine, acetylcodeine, heroin, noscapine, papaverine, propiophenone.
Opium sample elution order:	Morphine, procaine, codeine, meconic acid, thebaine, noscapine, papaverine.

NOTES

With minor modifications, the method can be adapted for the analysis of either heroin or opium. The method is particularly useful for raw opium, as there is no need to separate meconic acid before analysis. A limitation of this method is the long analysis time (1 hour). In return, however, the method provides good peak separation and peak shapes throughout the chromatogramme, while most common heroin adulterants (such as thiamine, procaine, caffeine, theophylline, and quinine) are fully resolved.

One concern which would be associated with any reverse phase method is the hydrolysis of heroin to form 6-acetylmorphine. In this method the injection solvent has been carefully chosen in order to minimize heroin hydrolysis (no significant hydrolysis should be detected for up to 16 hours); however, samples should be analyzed as soon as possible after dissolution in injection solvent. A second concern is a gradual degradation of separations with use as compared to those achieved with a new HPLC column. It has been found that the loss of resolution can be recovered, to a significant extent, by adding a small amount of hexylamine to the phosphate buffer. However, it is for this reason that the frequent incorporation of reference standards is required with 1for every 6 samples analyzed recommended.

The use of multi-wavelength detection, as suggested for this method, provides the analyst with two very significant advantages.[44] (1) If a chromatographic peak is composed of a single target compound, then each of the wavelengths measured will produce essentially identical quantification values. Hence, a significant deviation in these values indicates the probable presence of one or more co-eluting compounds. A significant enhancement of this technique is available when photo-diode array detectors are utilized, as these detectors allow for on-line observation of an entire UV spectrum, which in turn allows comparison of a spectrum taken on the up-slope with one taken on the down-slope of the chromatographic peak. (2) If the monitored wavelengths are chosen with care, the most frequently encountered co-eluting interferences either will not absorb, or will absorb very weakly at one or more of the monitored wavelengths, allowing for the successful quantification of the target compound.

The methods described above are but two of several available for the analysis of opium/heroin samples. Other HPLC methods which are designed for opiate analyses are described in the literature and several are offered as suggested reading.[45]

Finally, within the last few years much work has been performed using the relatively new technique of capillary electrophoresis (CE).[46] Several of these works have dealt with the analysis of opiates, and they have uniformly offered impressive separations in very short analysis times.[47] However, the technique is still relatively new, it is perhaps not yet as robust a technique as one would like, and experts are not yet in agreement as to the best methods for opiate analyses. For these reasons, CE is not recommended for the analysis of opiates at this point in time; however, it is also clear that this technique should be watched closely, and all are encouraged to explore the use of CE in their work.

CALCULATIONS

In general, if a chromatographic peak is well resolved and symmetrical, the peak area is the preferred measurement for quantification. Whether one uses peak height or peak area, the method for calculating the content of a target compound is the same, and is as follows:

$$C_{target}\% = \frac{C_{std}}{C_{sample}} X \frac{\frac{R_{target}}{R_{IS}}}{\frac{R_{std}}{R_{IS}}} X \frac{M_{target}}{M_{std}} X 100$$

- $C_{target}\%$ = the target compound expressed as percent in sample
- C_{std} = the standard reference compound expressed as weight/volume
- C_{sample} = the sample used in the analysis expressed as weight/volume
- R_{target} = response of the target compound
- R_{IS} = response of the internal standard
- R_{std} = response of the standard reference compound
- M_{target} = molecular weight of target compound*
- M_{std} = molecular weight of standard reference compound*

**If salt and hydrate forms of the target compound and the reference compound are the same then $M_{target}/M_{std} = 1$*

CALCULATION NOTES

The use of automatic procedures (calculation by computer) are encouraged, but it is still the responsibility of the analyst to ensure that the calculations are set up correctly.

For opium and morphine base samples, the amount of morphine should be reported as percent morphine base in the dried material. Morphine base is typically present as the monohydrate, while morphine hydrochloride is typically present as the trihydrate.

GENERAL QUANTITATION NOTES

The use of properly validated secondary standards are recommended as a considerable reduction in cost is achieved without any loss of analytical quality.[48]

Quantification methods which employ an internal standard are recommended for the quantification of opiate alkaloids.

Sampling errors are reduced if large aliquots of material are subjected to sequential dilution. If the cost of solvents presents no problem, and if the taking of a large aliquot will not significantly reduce the size of the exhibit to be taken to court, then this approach may be adopted. However, when large amounts of material are used for the first dissolution, it may be necessary to add the solvent by pipette in order to avoid errors due to the volume of insoluble materials. It is not common to find large amounts of insoluble materials in bulk quantities of heroin seized within source countries or at entry ports of developed countries, but considerable quantities of insoluble materials are the rule for raw opium, and occur frequently with "street" samples seized within all countries.

For each compound to be quantified, a calibration curve must be constructed. In no case should quantification results be accepted for an analyte quantity which is outside of the boundaries established by the associated calibration curve. For each order of magnitude change in concentration, a minimum of three points should be used to construct the calibration curve, with the minimum and maximum values being equally spaced above and below the "usual" quantity expected for the analyte. For example, the construction of a calibration curve over two orders of magnitude in sample concentration using GC-FID with a typical thin film narrow-bore capillary column might be accomplished by measuring a reference standard at 5 concentration values, such that **on-column loadings** of the reference standard are at 50, 25, 5, 2.5, and 0.5 ng where the "usual" column loading for the target compound in a sample would be expected to be 5 to 10 ng. In addition, a calibration curve must be verified with each use, and be re-constructed whenever the internal standard solution is changed. It is also important to remember that any change in column conditions will result in a corresponding change in the slope of the calibration line. In practice, verification of an existing calibration curve is usually accomplished by determining a single point on the curve (i.e., the calibration curve is assumed to be valid if the response ratio of a known quantity of reference standard and internal standard falls on the calibration line at the predicted point). If the sample and reference standard concentrations are kept within close proximity of each other, then this approach is suitable; however, if sample and standard concentrations are expected to differ substantially then a multi-point validation is required. Many laboratories require that a reference standard analysis be included with each sample analysis. In the absence of such a policy, it is recommended that at least two consecutively reproducible reference standard analyses precede the start of the analysis of the first sample, and as a minimum an additional reference standard be analyzed after every fifteen heroin samples, or after every eight opium samples. It should also be recognized that some chromatographic

systems can require more frequent analysis of the reference standard, (i.e.,HPLC method B requires a minimum of one reference standard analysis for every 6 samples). Additionally, the analyst should include an appropriate number of negative standard (blank) samples in order to assure that there are no phantom responses for either the target compound or the internal standard. For the consecutive analysis of samples and reference standards numbering less than or equal to fifteen, a blank should be included at the beginning and end of the analytical run; if larger than fifteen, an additional blank should be inserted in the middle of the analytical run.

An accurate quantitative analysis, using either HPLC or GC, depends upon the proper functioning of the chromatographic system. Therefore, it is incumbent upon the analyst to ensure that the system is operating properly. Typically, the instrument manufacturer has a set of specifications that utilize one or more test solutions. When these specifications are met, proper instrument function is implied. However, rather than use the manufacturer's test solutions, it is frequently more useful for the forensic analyst to use a test solution containing a set of target compounds, (e.g., for an opium analysis, a solution containing morphine, codeine, thebaine, papaverine, and noscapine).

SPECTROSCOPIC/SPECTROMETRIC DATA

INFRARED SPECTROSCOPY

Sample Preparation

The preferred methods of sample preparation are generally considered to be dissolution of the sample in an appropriate solvent, or suspension of the sample in an oil mull. A less favoured technique is the halide disk method where the sample is dispersed into finely ground potassium halide (KBr or KCl) and pressed into a thin disk. However, most forensic laboratories favour the halide disk method for two reasons: (1) potassium halides are IR transparent in the so-called finger print region (2000-400 cm^{-1}); and (2) the potassium halide disk can generally be stored and re-analyzed many times if the disks are stored over a desiccant.

The halide disk method consists of mixing approximately 1 mg of dry sample with about 200 mg of an alkali halide, and grinding the mixture until it is a fine powder; after grinding, the mixture is pressed into a thin, transparent disk. Both KCl and KBr work equally well; however, KCl is slightly less hygroscopic, and is generally recommended over KBr, especially when the analyte is a hydrochloride salt. Failure to grind the sample thoroughly can result in small shifts in absorbance bands, both in magnitude and wavelength, from the normally accepted values.[49] A number of commercially available disk pressing systems are available today, some of which are quite inexpensive. Whether KBr or KCl is used, it should be "IR grade" and dried at 110 °C for a minimum of one hour. It can be stored above a strong desiccant (e.g., phosphorus pentoxide) in a desiccator, or left in the oven and removed on an "as needed" basis. This may be important in any subsequent legal proceeding. Also, the material under investigation can be recovered form the halide disc for further testing.

A potential problem with the use of KBr is the production of an erroneous spectrum due to halide exchange with hydrochloride salts during preparation of the disc:

$$Base{\bullet}HCl + KBr \rightarrow Base{\bullet}HBr + KCl$$

A frequent culprit in this conversion is the use of excessive pressure when grinding the sample-KBr mixture. Excessive grinding pressure can also result in the production of polymorphic forms. When grinding sample in an agate mortar use a light pressure. Holding the pestle with only two fingers usually gives good results. The use of potassium chloride, rather than potassium bromide, effectively eliminates the halide exchange problem for the analysis of hydrochloride salts.

An additional, minor disadvantage to the halide disk method is that the potassium halides are hygroscopic. Therefore, the acquisition of a spectrum which does not show the free hydroxyl band of water can be problematic.

The micro halide disc method involves the use of commercially available dies which can produce a halide disc only 1 mm in diameter. The quantity of halide required is reduced approximately 100 fold, as is the amount of sample needed. The use of this technique finds particular application in the analysis of components that have been eluted from TLC plates or analytical HPLC systems.

The Nujol mull method requires the mixing of a finely powdered sample (2-3 mg) with one drop of nujol (liquid paraffin or perfluorinated long-chain alkane), and then grinding the mixture in an agate mortar. The quantity of nujol added is adjusted so that the final mull is the consistency of a thin cream. The resulting mull is spread on an alkali halide disc (usually KBr) and a similar disc placed on top. The film between the halide discs should contain no air bubbles. The obvious disadvantage of this method is interference from the Nujol in the spectrum.

INFRARED SPECTRA AND TABULAR DATA

The following infrared spectra were prepared by the potassium halide method using 1 mg of sample in approximately 200 mg of KBr. The spectra were prepared from reference standards, all of which were free bases. A spectrum of morphine base for the monohydrate and anhydrous forms are provided in Figures I and II, respectively. Anhydrous morphine base was prepared by heating the monohydrate for 1 hour at 115 °C. All other standards were utilized "as is."

FIGURE I.	INFRARED SPECTRUM OF MORPHINE BASE MONOHYDRATE
FIGURE II.	INFRARED SPECTRUM OF MORPHINE BASE
FIGURE III.	INFRARED SPECTRUM OF HEROIN BASE
FIGURE IV.	INFRARED SPECTRUM OF CODEINE BASE MONOHYDRATE
FIGURE V.	INFRARED SPECTRUM OF ACETYLCODEINE BASE
FIGURE VI.	INFRARED SPECTRUM OF THEBAINE BASE
FIGURE VII.	INFRARED SPECTRUM OF PAPAVERINE BASE
FIGURE VIII.	INFRARED SPECTRUM OF NOSCAPINE BASE

FIGURE I. INFRARED SPECTRUM OF MORPHINE BASE MONOHYDRATE

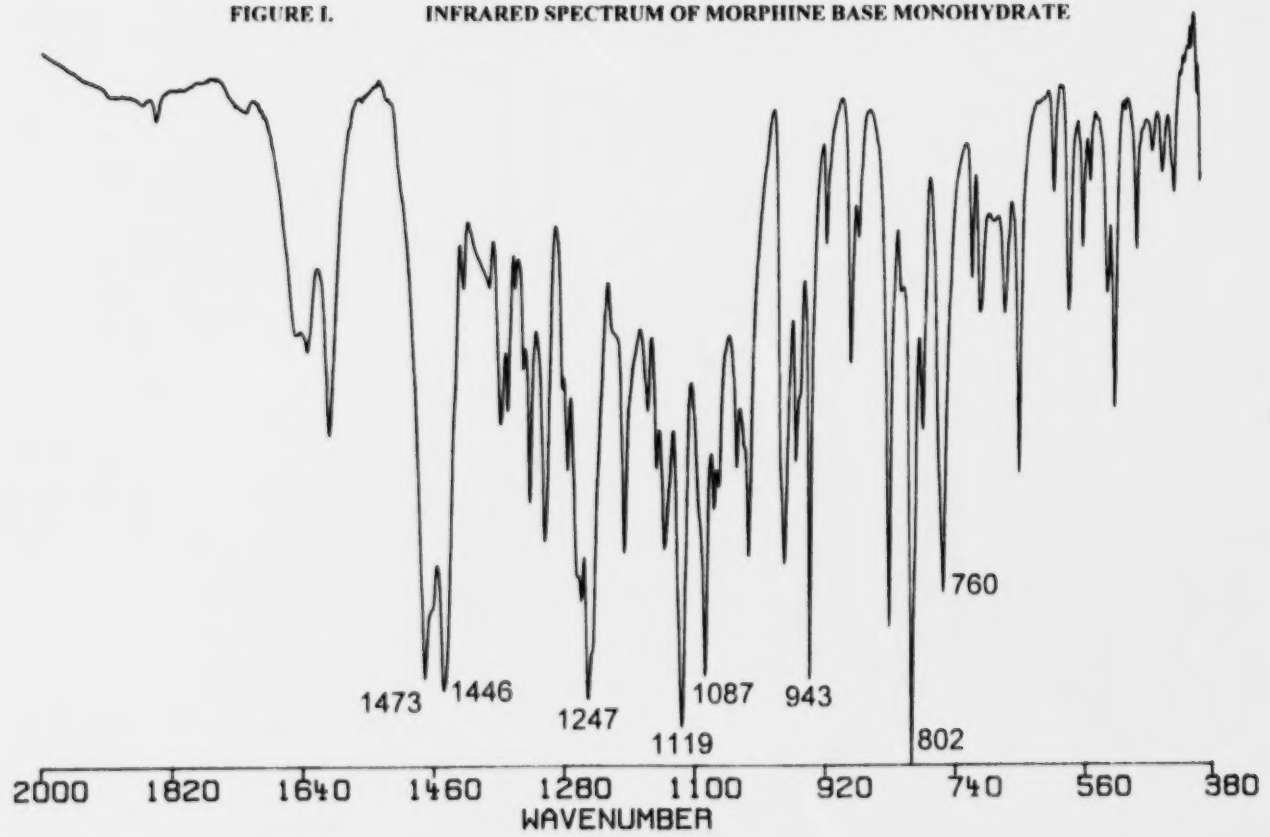


FIGURE II. INFRARED SPECTRUM OF MORPHINE BASE

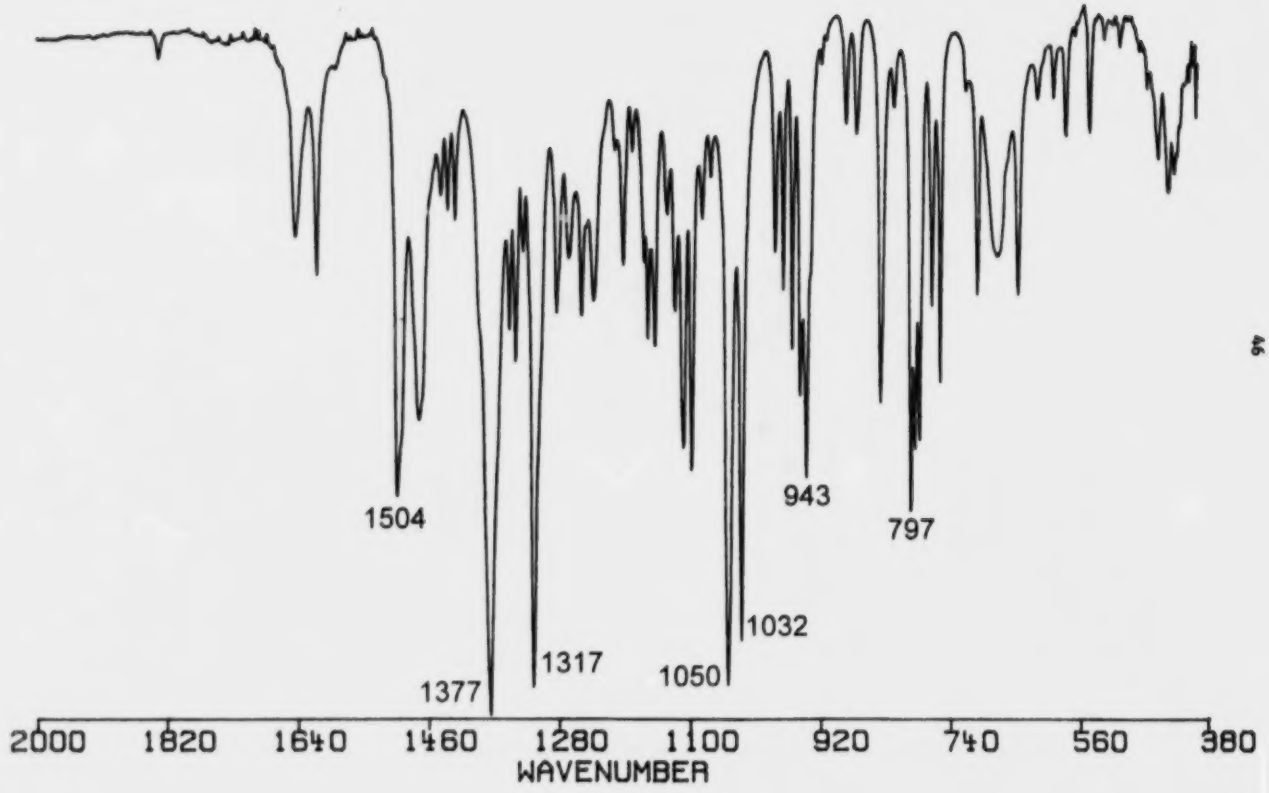


FIGURE III. INFRARED SPECTRUM OF HEROIN BASE

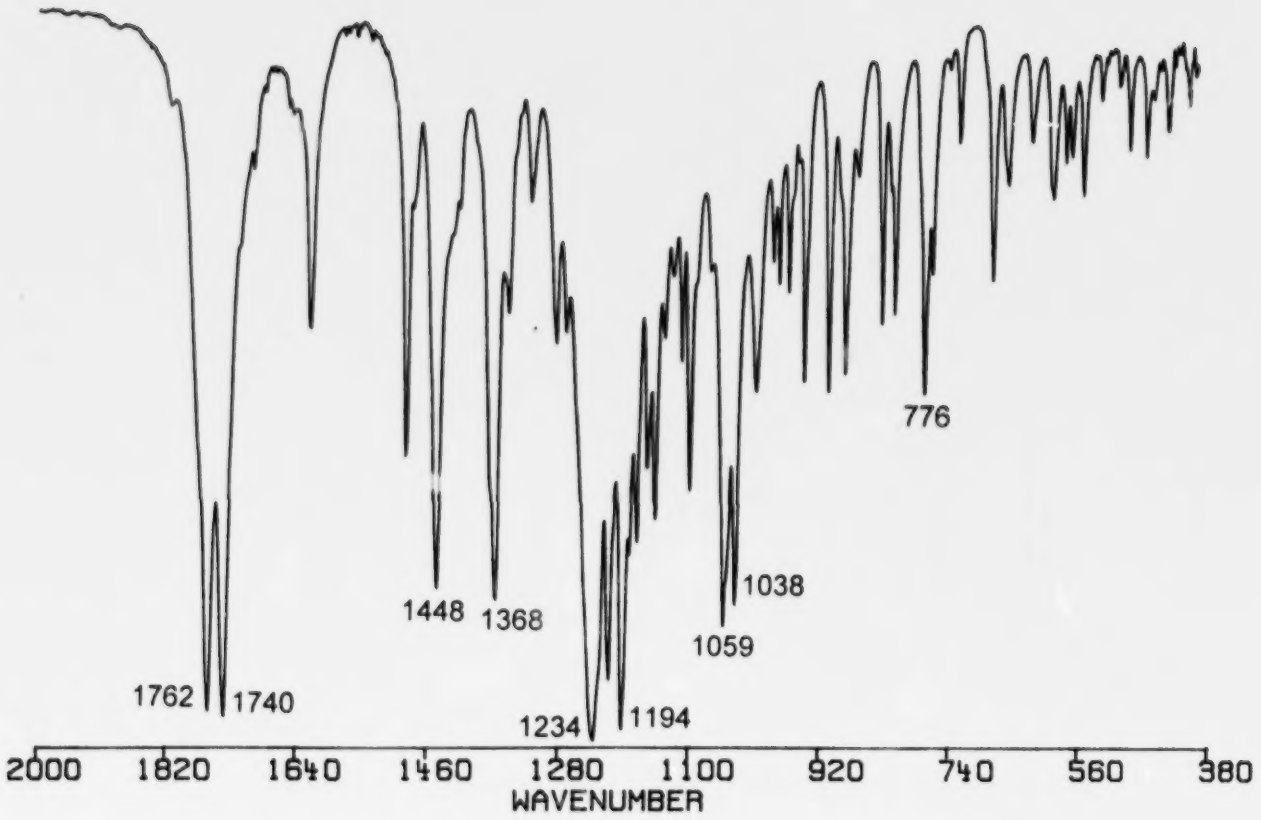
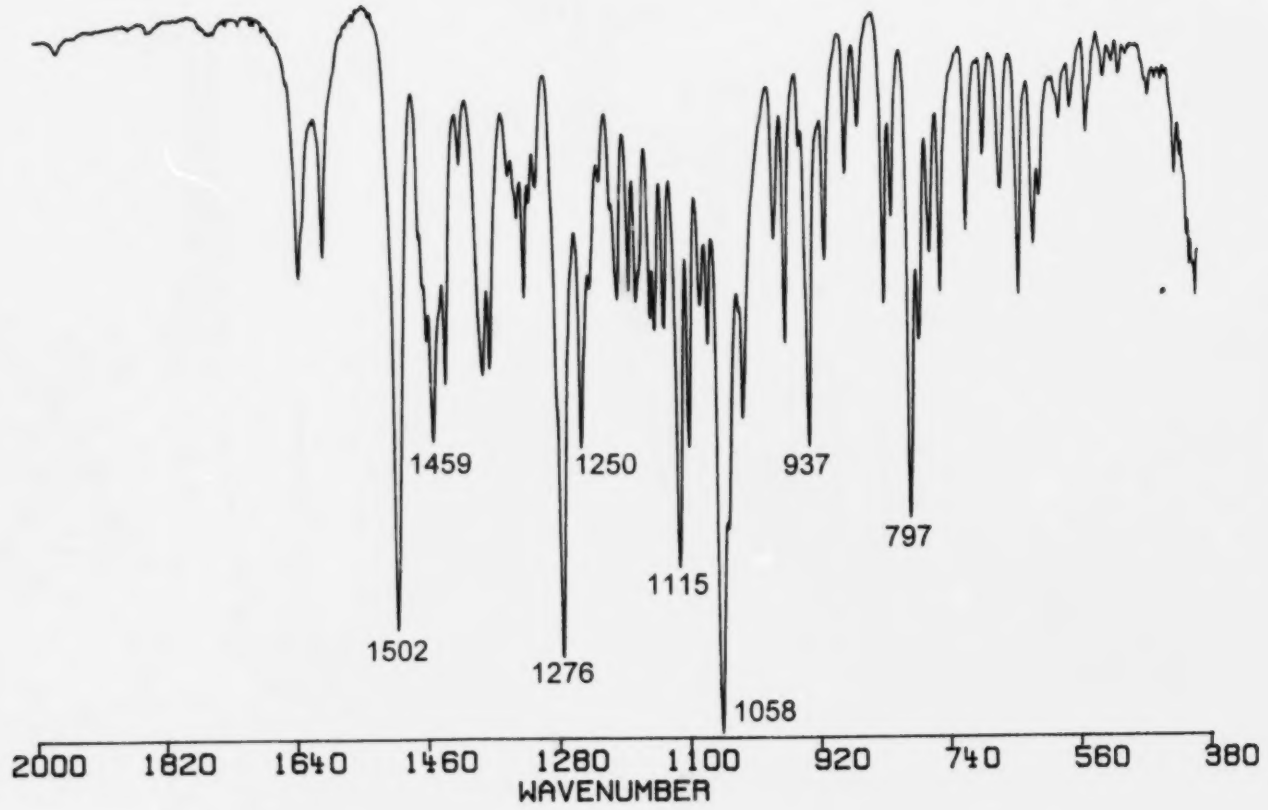


FIGURE IV. INFRARED SPECTRUM OF CODEINE BASE MONOHYDRATE



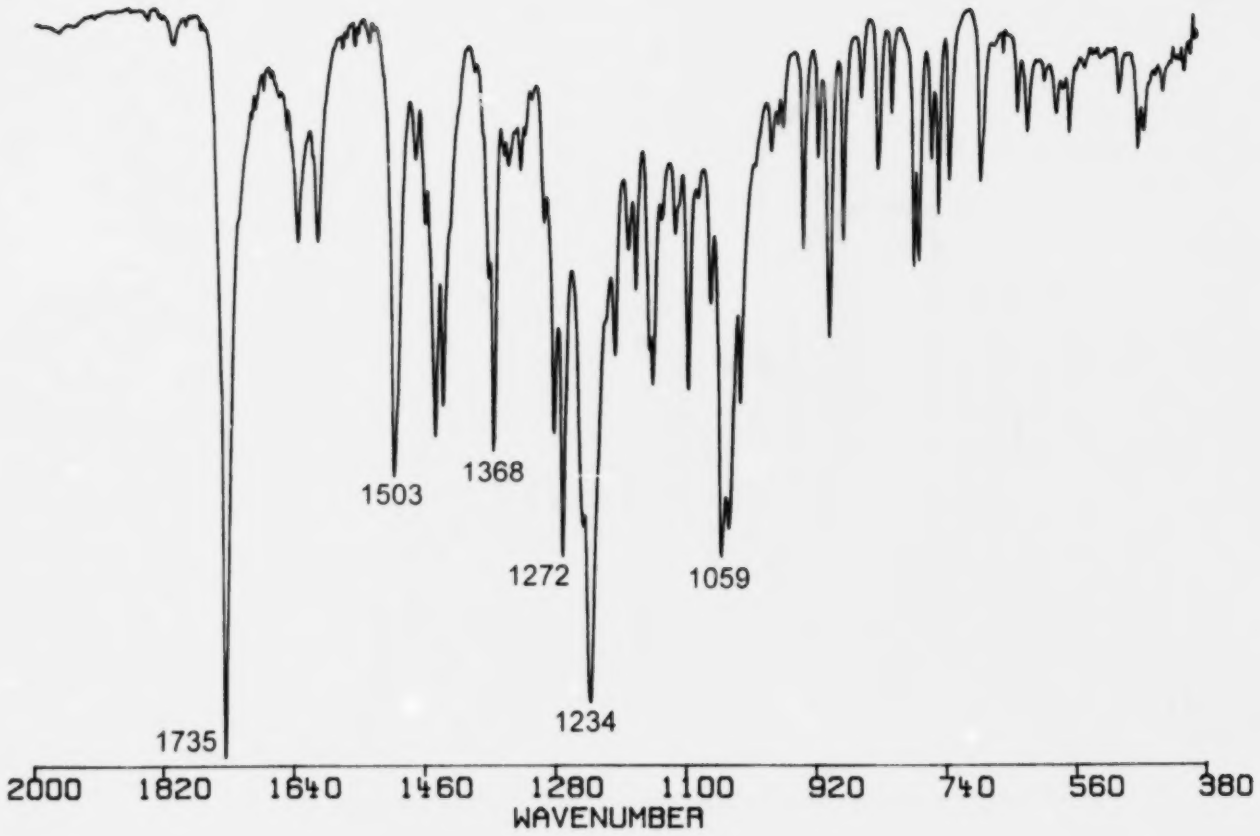

1735
1503
1368
1272
1234
1059
2000
1820
1640
1460
1280
1100
920
740
560
380
WAVENUMBER

FIGURE VI. INFRARED SPECTRUM OF THEBAINE BASE

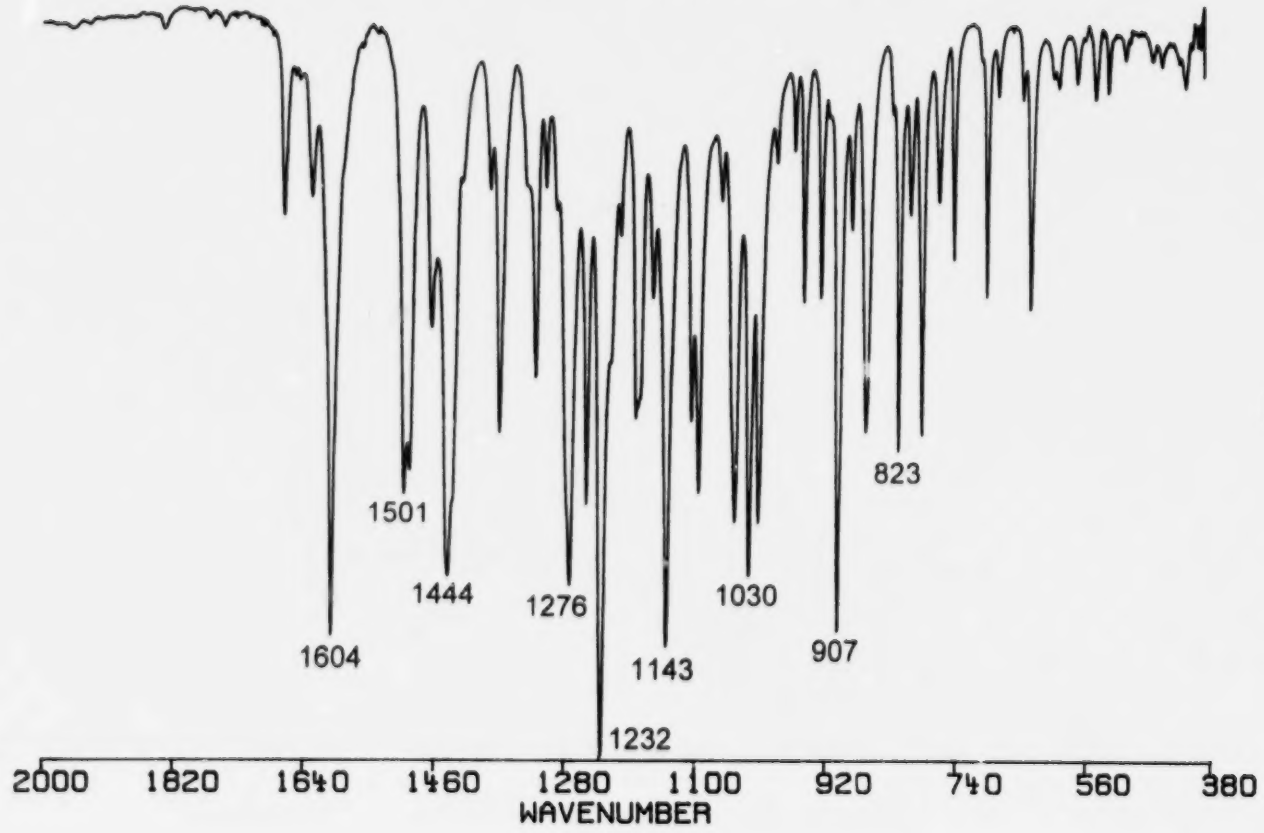


FIGURE VII. INFRARED SPECTRUM OF PAPAVERINE BASE

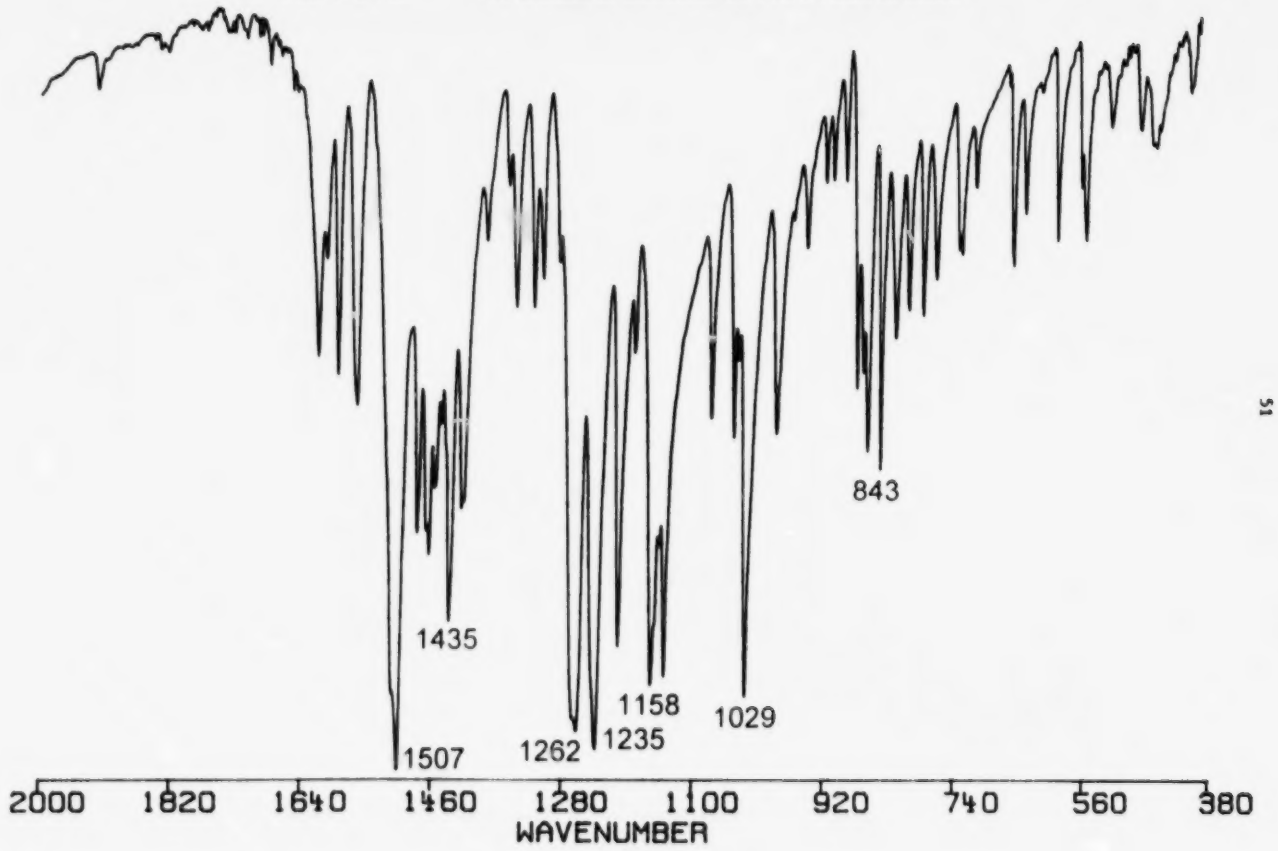
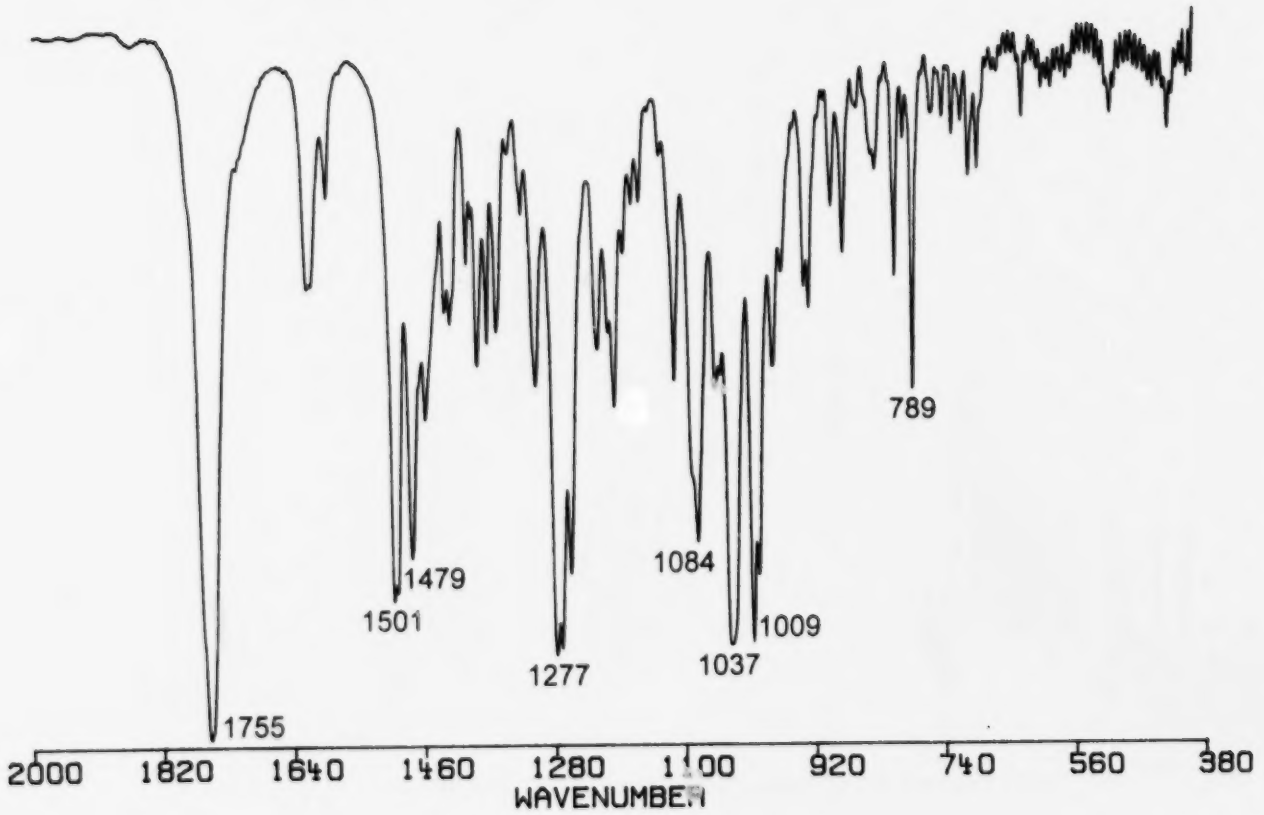


FIGURE VIII. INFRARED SPECTRUM OF NOSCAPINE BASE



The IR data in the following table are provided as an adjunct to the preceding spectra. Standards were prepared for analysis by the halide disk method using KBr. Major peaks for the listed compounds are given in descending order of magnitude of absorbance.

TABLE IX[50]

Opiate IR Absorbance in cm^{-1} Decreasing Order of Magnitude								
Heroin Base	1243	1196	1727	1214	1444	1757	1054	1370
Heroin HCl	1245	1736	1177	1194	1448	1765	1157	1368
Morphine Base	802	1244	1445	1117	941	1468	759	1086
Morphine HCl	1444	1224	787	1409	1449	1460	1076	------
Morphine H_2SO_4	1270	1640	1520	1470	1330	1120	1080	970
Codeine Base	1059	1277	1501	1116	797	1252	938	------
Codeine HCl	1442	784	1408	1456	1491	1111	1123	------
Codeine H_2SO_4	1110	1063	1039	1443	1496	1267	612	784
6-Acetylmorphine Base	1239	1740	1018	1038	1374	1459	1505	915
6-Acetylmorphine HCl	1240	1723	1503	1039	1305	1368	1465	805
Acetylcodeine Base	1238	1739	1057	1277	1505	1455	1290	1375
Acetylcodeine HCl	1241	1739	1052	1509	1445	1372	1118	910
Papaverine Base	1508	1262	1239	1159	1031	1141s	1438	1205
Papaverine HCl	1510	1282	1265s	1410	1435	1028	1243	1148
Noscapine Base	1759	1279	1039	1504	1009	1482	1261s	1085

NOTES

There exists more than one crystal and hydrate form for both morphine and heroin.[51] As a result, it is imperative that each laboratory prepare a standard reference spectra library where the standard reference compounds have been treated exactly in the same manner as the corresponding samples. All compounds identified by IR and reported by the analyst must be compared to an IR spectrum of the appropriate reference standard obtained from the same instrument under the same operational conditions as the reported compound.

MASS SPECTROMETRY

Without question the GC-interfaced mass spectrometer (GC/MS) is one of the most useful tools available to the forensic drug chemist. Operating under electron ionization conditions, the modern computer-dedicated GC/MS is capable of unattended analysis of multiple samples, with analyte sensitivities near those of a GC using flame ionization detection, and with analyte specificity near that of infrared spectroscopy. However, it is the ability to provide highly specific spectral data on individual compounds in a complex mixture of compounds, and to do so without prior separation of these components, that is the true value of GC/MS.

GC/MS METHOD

Operating Conditions

Column:	Fused silica, 30 m x 0.25 mm I.D. with 0.25 µm crosslinked 100% Methyl Siloxane stationary phase (directly coupled to the mass spectrometer)	
Carrier Gas:	He set to 50 cm/sec. at 220 °C	
Injection technique:	20:1 Split	
Injection volume:	1 µl	
Temperatures:	Injector	260 °C
	GC/MS Interface	275 °C
	MS source	180 °C*
	Oven Temperature programme	
	1.) 175 °C, with 1 min. hold time.	
	2.) 6 °C/min. to 300 °C	
	3.) End of programme	
Scan parameters:	34-450 Daltons in approximately 1 second	
Ionization mode:	EI at 70 eV	

* *Some instruments do not allow for adjustment of source temperature.*

Sample Preparation

Opium samples may be prepared as stipulated in the previously provided GC methods. Uncut heroin and morphine samples can be directly derivatized by adding approximately 10 µl of BSA and 1 ml of methylene chloride per 0.5 mg of sample to a dry glass container, and heating at 60 °C for 20 minutes. In general, the GC/MS analysis of morphine should be accomplished after derivatization. Although heroin does not derivatize using BSA, there often are other components mixed with the heroin which do derivatize, and whose chromatographic behaviors are enhanced by derivatization. Some analysts prefer to analyze heroin samples without prior derivatization, and this is generally an acceptable approach. If direct dissolution of the sample into injection solvent is performed, then a solvent should be selected which will exclude sugars and salts other than organic halide salts. A solvent consisting of 4:1 methanol-methylene chloride (or chloroform) will dissolve most known adulterants or controlled substances found in heroin samples, while excluding sugars; however, this approach may not be suitable for quantitation.

Mass Spectra

The following mass spectra are provided for user reference only. All compounds identified by GC/MS and reported by the analyst must be compared to a current mass spectrum of the appropriate reference standard obtained from the same instrument operated under the same conditions. There are several commercial mass spectra reference libraries available, with one or more of the most extensive libraries available as an option for most mass spectrometers. It is especially important to use mass spectral libraries, whether from a commercial source or user generated, for reference purposes only. A single source for mass spectral interpretation of the opium alkaloids is not available, but there are several good literature resources[52] which in total are reasonably comprehensive.

FIGURE IX.	EI MASS SPECTRUM OF 3,6-DI-(TRIMETHYLSILYL) MORPHINE
FIGURE X.	EI MASS SPECTRUM OF HEROIN
FIGURE XI.	EI MASS SPECTRUM OF 3-TRIMETHYLSILYL-6-ACETYLMORPHINE
FIGURE XII.	EI MASS SPECTRUM OF 3-ACETYL-6-TRIMETHYLSILYLMORPHINE
FIGURE XIII.	EI MASS SPECTRUM OF TRIMETHYLSILYLCODEINE
FIGURE XIV.	EI MASS SPECTRUM OF ACETYLCODEINE
FIGURE XV.	EI MASS SPECTRUM OF PAPAVERINE
FIGURE XVI.	EI MASS SPECTRUM OF NOSCAPINE
FIGURE XVII.	EI MASS SPECTRUM OF THE TRI-TMS DERIVATIVE OF MECONIC ACID

FIGURE IX. **EI MASS SPECTRUM OF 3,6-DI-(TRIMETHYLSILYL) MORPHINE**

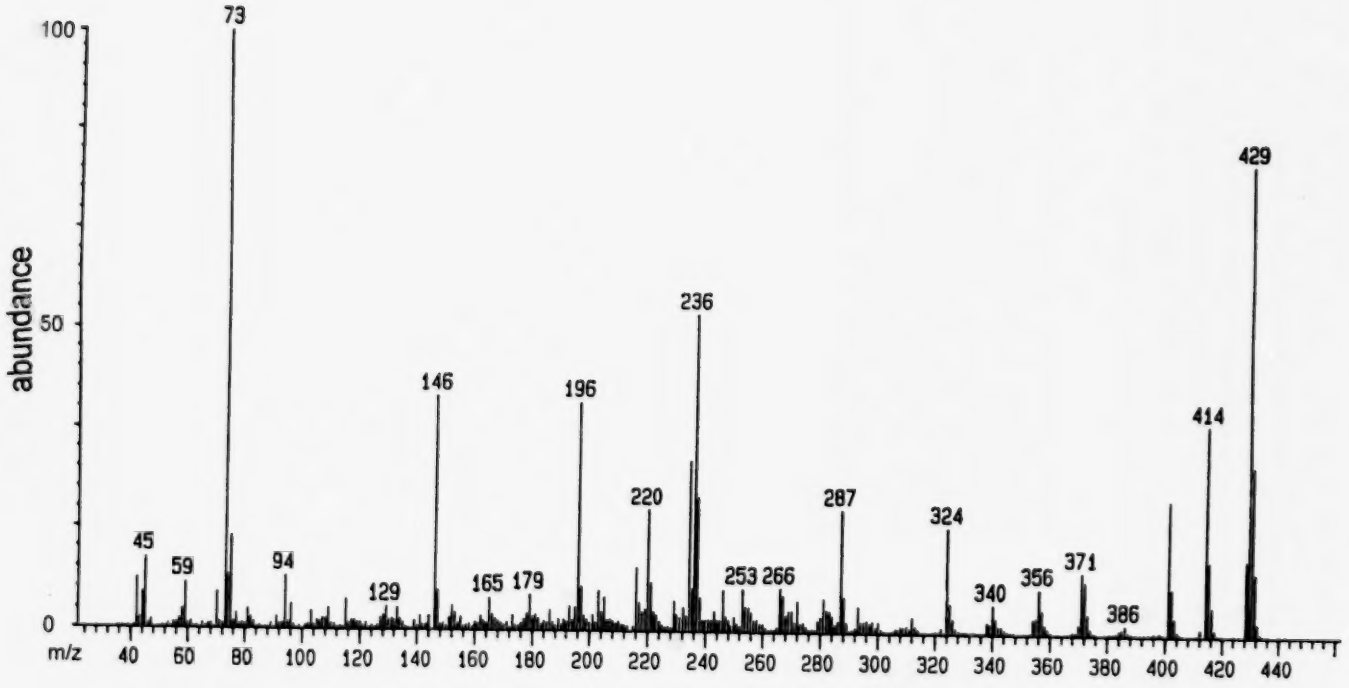


FIGURE X. EI MASS SPECTRUM OF HEROIN

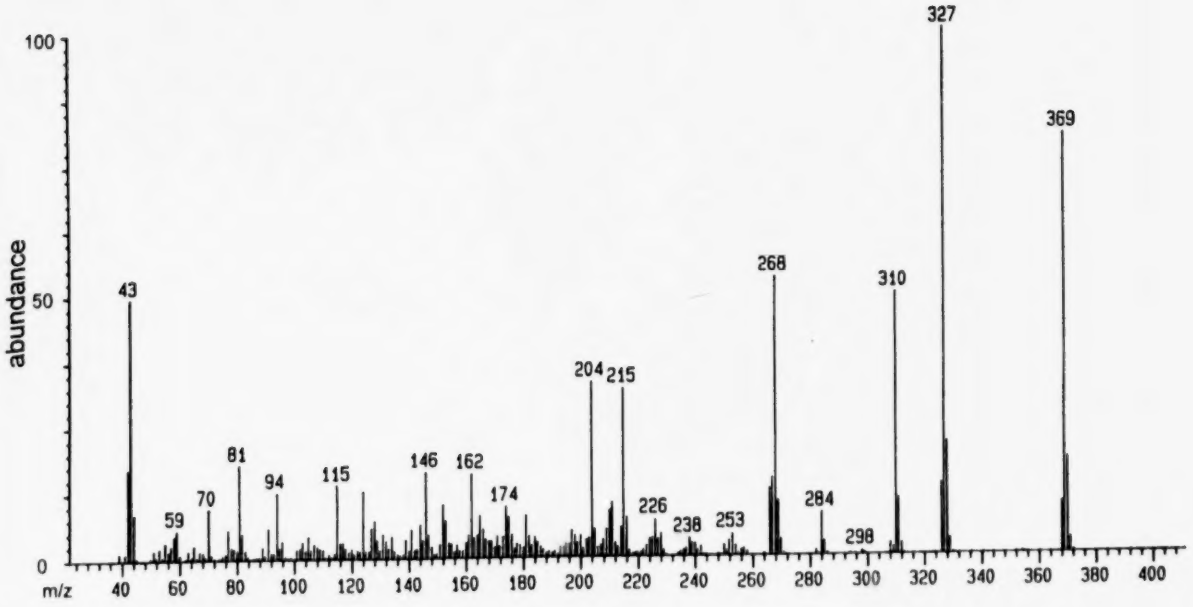


FIGURE XI. **EI MASS SPECTRUM OF 3-TRIMETHYLSILYL-6-ACETYLMORPHINE**

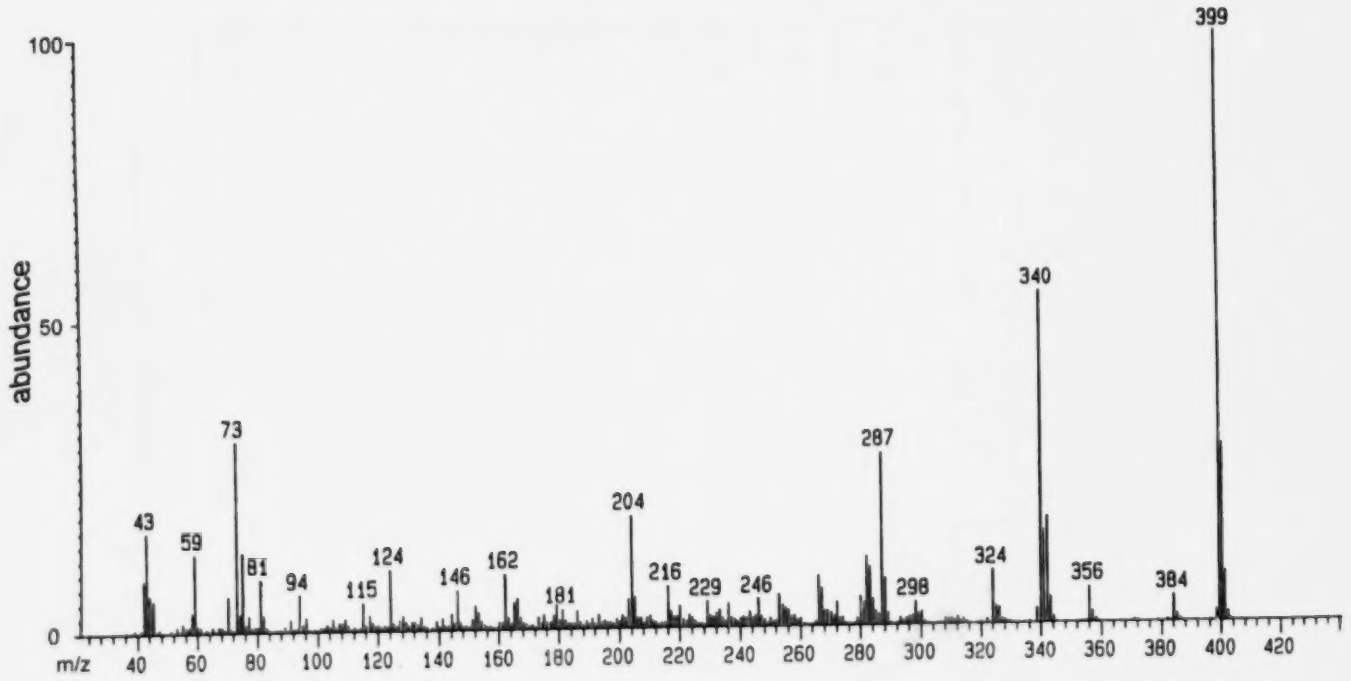


FIGURE XII. **EI MASS SPECTRUM OF 3-ACETYL-6-TRIMETHYLSILYLMORPHINE**

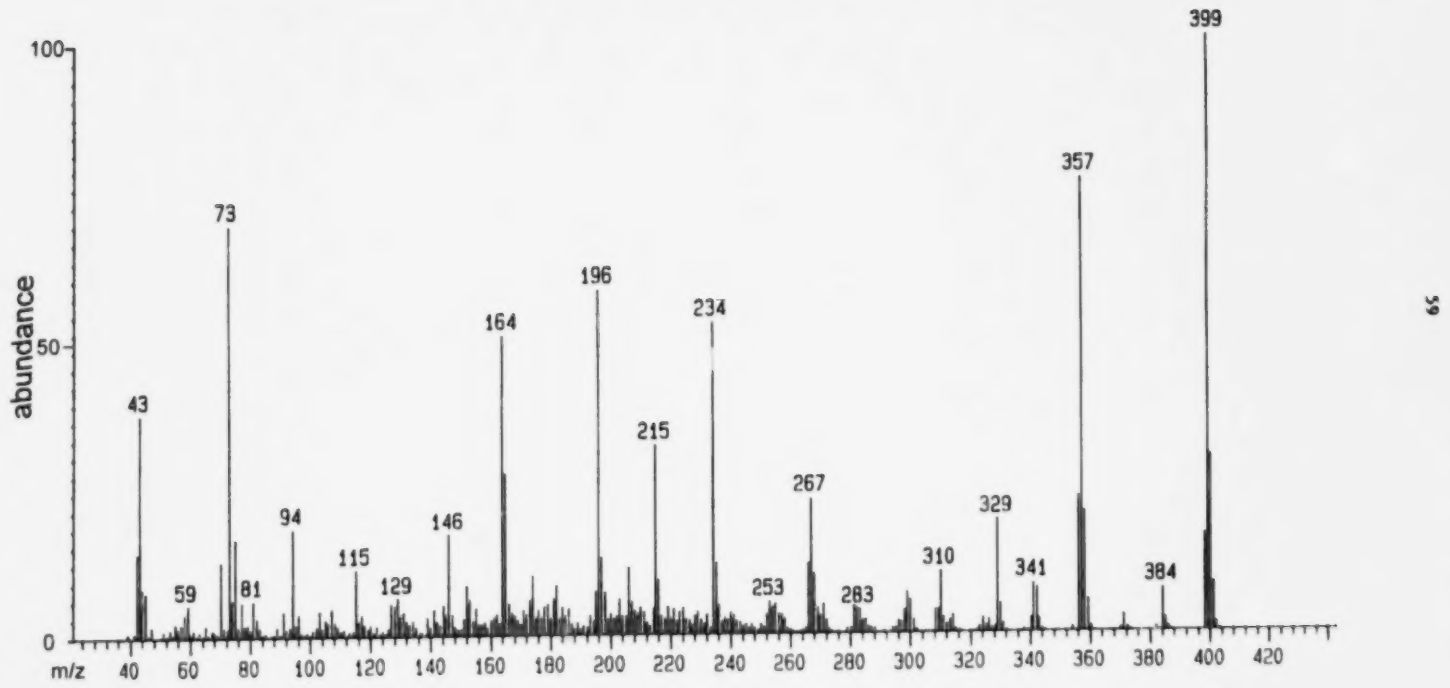


FIGURE XIII. EI MASS SPECTRUM OF TRIMETHYLSILYLCODEINE

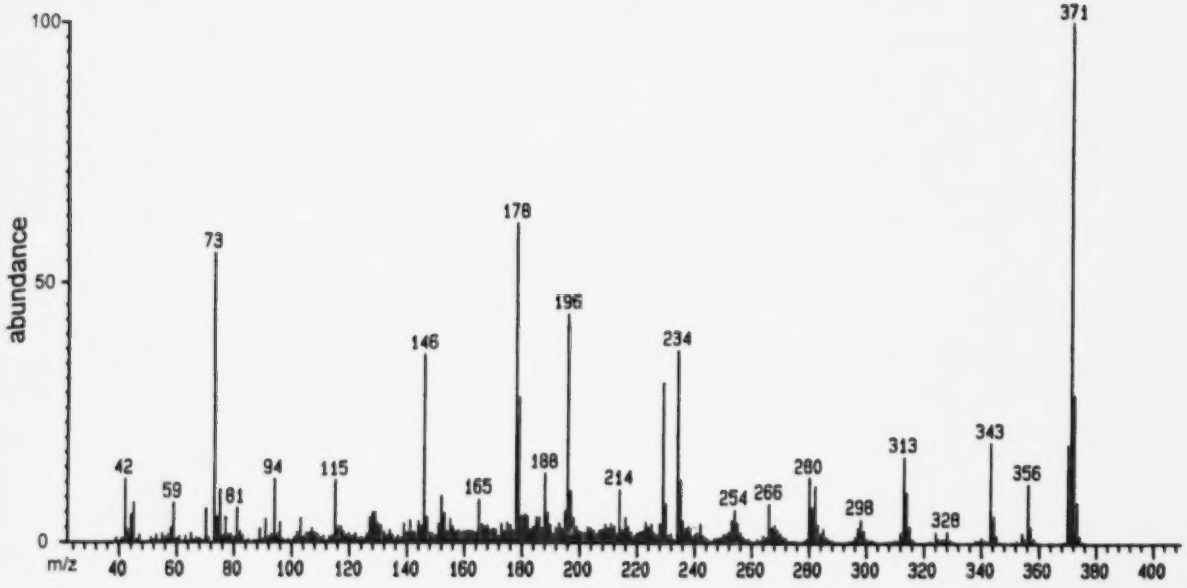


FIGURE XIV. EI MASS SPECTRUM OF ACETYLCODEINE

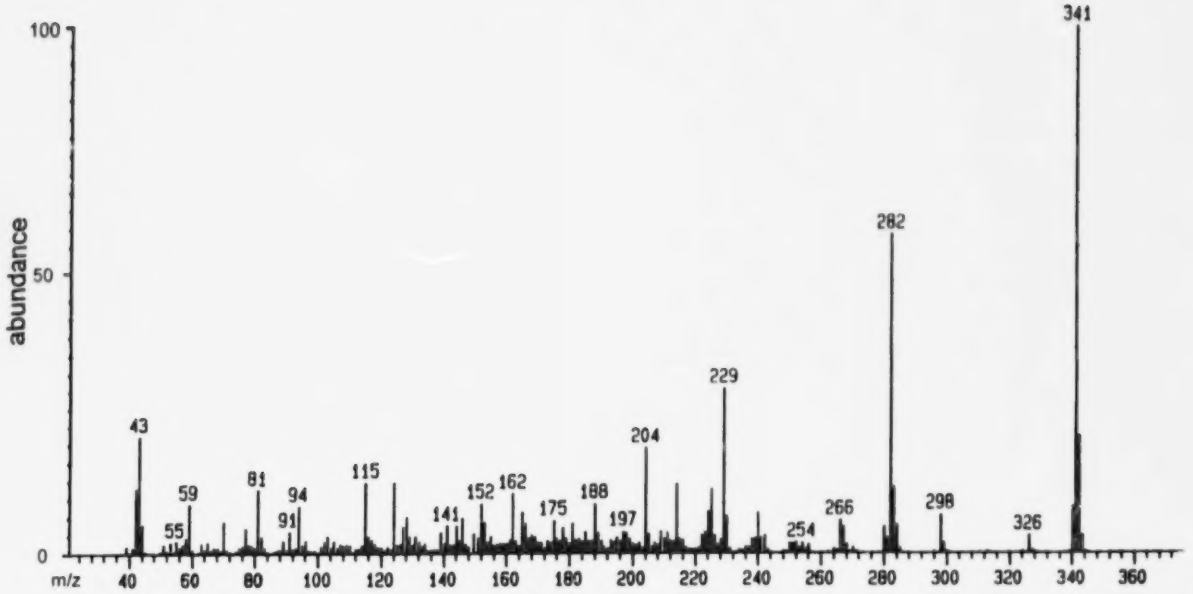


FIGURE XV. EI MASS SPECTRUM OF PAPAVERINE

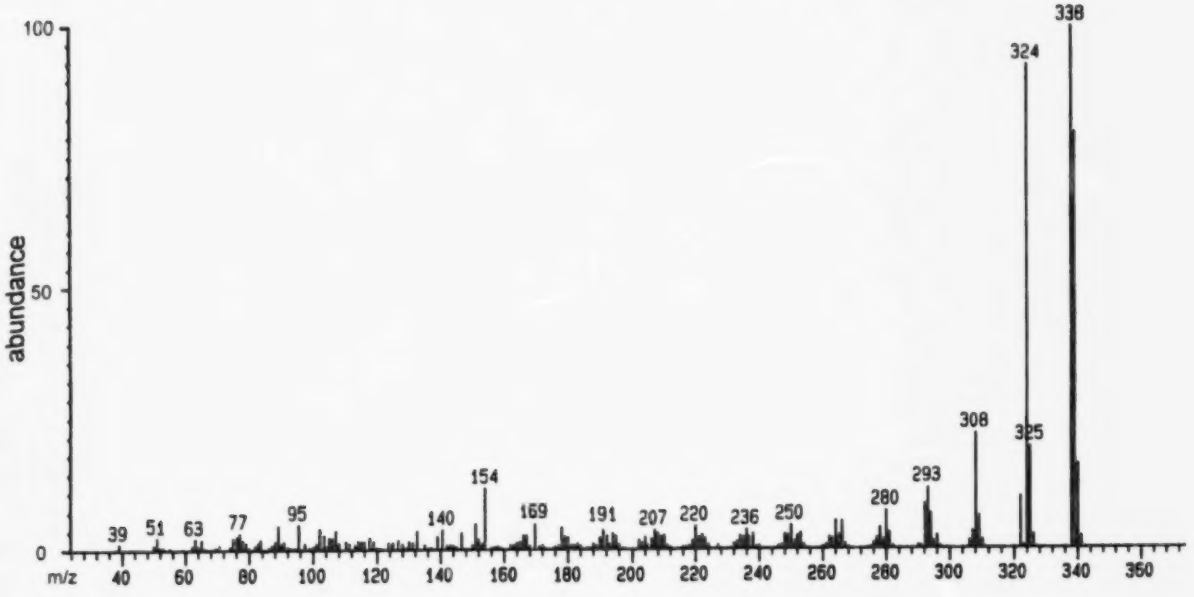


FIGURE XVI. EI MASS SPECTRUM OF NOSCAPINE

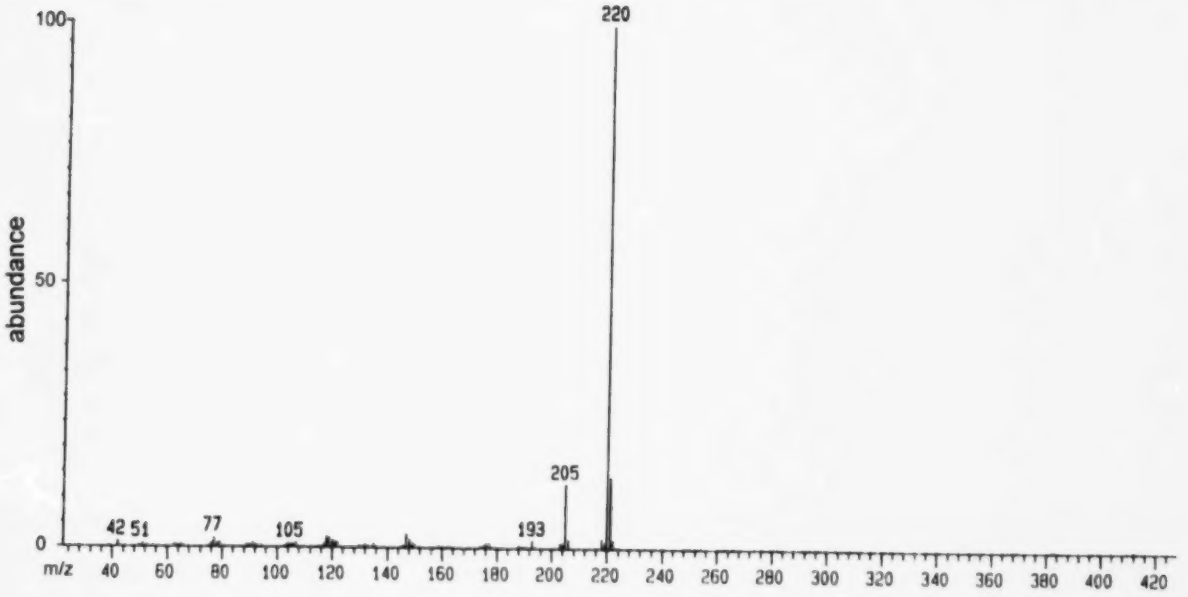
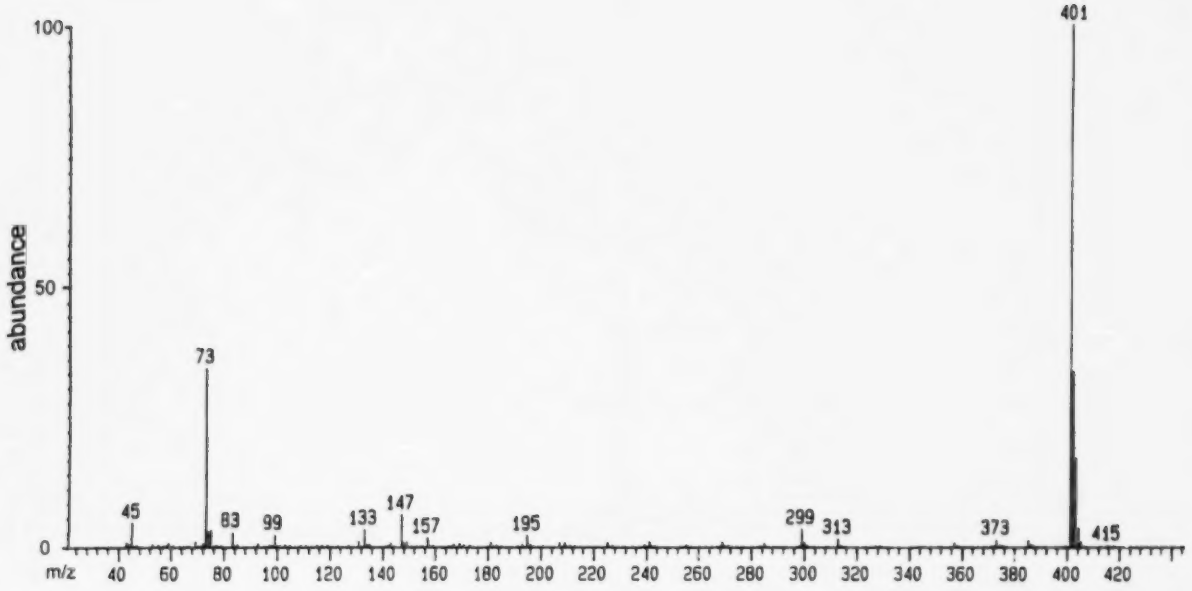


FIGURE XVII. **EI MASS SPECTRUM OF THE TRI-TMS DERIVATIVE OF MECONIC ACID**



NOTES

The gas chromatograph interfaced mass spectrometer (GC/MS) suffers from the same limitations as those described in the previous section on GC methods. Therefore, it is equally important to maintain the GC, with particular emphasis placed upon the injection port.

A GC/MS is very conservative of sample, as only milligramme quantities are typically taken for analysis and, if necessary, all but a few microgrammes of the sample material can be retrieved for other purposes. However, one must remember that those few unretrievable microgrammes are destroyed inside the mass spectrometer, and that some portion of the resulting residues from this process are left inside the instrument as contaminants. Fortunately, these contaminants adhere tightly to the interior components of the instrument, and lend minimally to instrumental background noise levels. Unfortunately, as these contaminants gradually coat ion steering surfaces, instrument performance deteriorates (generally most noticeable as a loss in response for ions greater than 250 Daltons); hence, the abundance of higher mass ions relative to low mass ions will decrease with instrument usage. Under normal operational conditions, this change in instrument performance and the resulting change in the spectral data occurs very slowly, and is not noticeable in day-to-day operation. Nevertheless, the operative concept is that the spectral data will be changed by the analysis process itself, and as a result, it is imperative that reference standard spectral data be acquired frequently, at a minimum, once every day. A corollary to the preceding discussion is that this inevitable degradation of mass spectrometer performance can be minimized by limiting sample quantities injected to the minimum consistent with the generation of reproducible spectra. This minimum quantity is highly instrument specific, and should be determined for each mass spectrometer. However, a coarse guideline for analyte minimum quantities can be provided for use with a narrow bore capillary GC/MS operating under electron ionization conditions. Typically, for an instrument with good sensitivity, the minimum amount of analyte required to give reproducible spectra occurs when the average quantity of analyte entering the source is between 0.2 and 1 ng/sec. Those instruments which are least sensitive may require an influx of sample into the source of as much as 20 ng/sec.

ANNEX I

DEFINITIONS

ALKALOID	An organic nitrogenous compound of plant origin.
EPICARP	The seed capsule is contained by the pericarp. The pericarp is composed of variously modified cells with the outermost layer being composed of a band of tightly packed cells. This layer is known as the epicarp.
LATICIFEROUS VESSELS	Vessels or sacs within a plant which are formed of specialized cells which produce latex. These structures in the opium poppy are more properly referred to as "articulated lacticifers" and are composed of longitudinal chains of cells which do not have a wall between the individual cells.
MEDICINAL OPIUM	A dried opium which has been reduced to a fine or moderately fine powder, whose morphine content has been adjusted to the parmacopoeial requirement of 9.5-10.5% (9.8-10.2% in some countries) by the addition of powdered lactose, cocoa husk, or rice starch. It is usually a light shade of brown and has the characteristic smell of opium.
OPIATES	Substances derived from opium poppy (*Papaver somniferum*) such as morphine, codeine, including their derivatives, such as heroin.
OPIUM	The air-dried milky latex obtained by incision of the seed capsules of *Papaver somniferum*.
OPIUM ALKALOID	An alkaloid obtained from opium.
OPIUM DROSS	The product that remains in the pipe after opium has been smoked.
PREPARED OPIUM	A product prepared from raw opium for the purpose of smoking.
RAW OPIUM	Opium latex which has been air dried to form a sticky tar-like brown gum

ANNEX II

REAGENT PREPARATION

1. *Marquis reagent*: Add 8-10 drops of 40% formaldehyde solution to 10 ml of concentrated sulfuric acid.

2. *Mecke's reagent*: Dissolve 0.25 gm of selenious acid in 25 ml of concentrated sulfuric acid.

3. *Frohde's reagent*: Dissolve 50 mg of either molybdic acid or sodium molydate in 10 ml of hot concentrated sulfuric acid. The freshly prepared reagent should be colourless.

4. *Dragendorff's reagent*: Solution (1) - Mix 2 gm of bismuth subnitrate, 25 ml of glacial acetic acid, and 100 ml of water.
Solution (2) - Dissolve 40 gm of potassium iodide in 10 ml of water.
Mix 10 ml of (1), 10 ml of (2), 20 ml glacial acetic acid, and 100 ml of water to produce Dragendorff's reagent.

5. *Acidified potassium iodoplatinate reagent*: Dissolve 0.25 g of platinic chloride and 5 g of potassium iodide in 100 ml of water. Add 2 ml of concentrated hydrochloric acid to create the acidified version.

6. *Ferric chloride solution*: Dissolve 1.0 gm of ferric chloride in 10 ml of water.

7. *Silver nitrate solution*: Dissolve 0.5 gm of silver nitrate in 10 ml of water.

8. *Barium chloride solution*: Dissolve 1.0 gm of barium chloride in 10 ml of water.

9. *Dilute Ammonia solution*: Dilute 375 ml of concentrated ammonia solution to 1 liter.

10. *Congo Red solution*: Dissolve 0.01 gm of Congo Red in 10 ml of water.

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